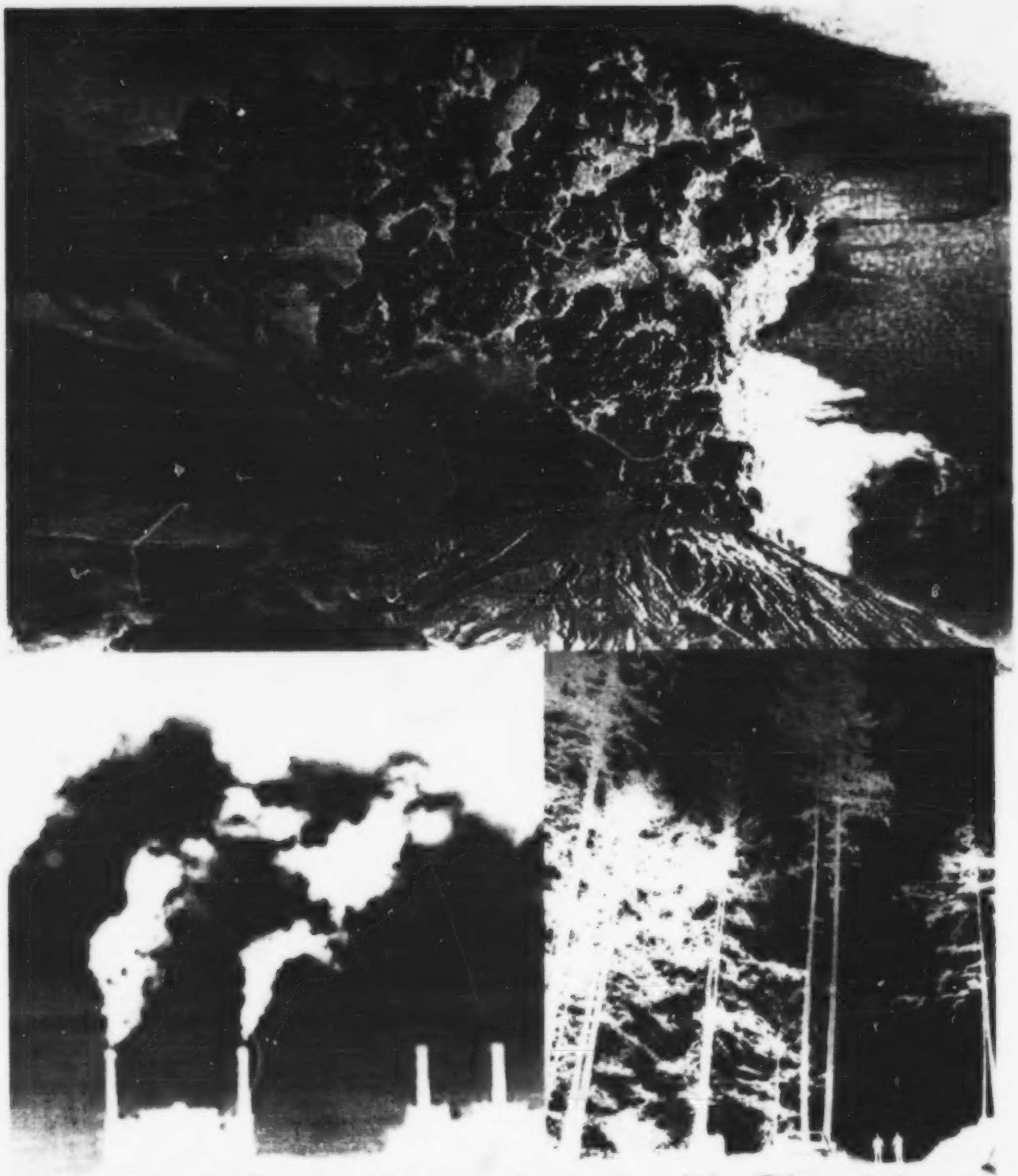

REPORT

Health Effects Associated with Short-term Exposure to Low Levels of Sulphur Dioxide (SO_2) - A Technical Review

Alberta Health and Wellness

2006

Health Surveillance

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HEALTH EFFECTS ASSOCIATED WITH SHORT-TERM EXPOSURE TO LOW LEVELS OF SULPHUR DIOXIDE (SO_2)

-A TECHNICAL REVIEW-

Alberta Health and Wellness
Health Surveillance
Health Strategies Division

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EXECUTIVE SUMMARY

In response to Recommendations 9 and 59 of the final report of the Provincial Advisory Committee on Public Safety and Sour Gas released in December 2000, Alberta Health and Wellness commissioned reports on the health effects of low-level exposure to hydrogen sulphide (H_2S) and sulphur dioxide (SO_2). The H_2S report on short-term exposure was released in July 2002 (Cantox Environmental, 2002). The present report on SO_2 is the second of four reports. The goal of these reports was to provide a comprehensive review of the available primary scientific literature in order to develop a quantitative understanding of the current state of knowledge with respect to the dose-response relationship between exposure to these contaminants (H_2S and SO_2) and health effects based on the weight of evidence in the peer-reviewed scientific literature. The focus of the third and fourth reports will be on the health effects of chronic exposure to H_2S and SO_2.

The development of the Terms of Reference of the H_2S report was undertaken by an expert panel over a six-month period. The format and goal of this SO_2 report was much the same as the previously completed H_2S report. In addition the Terms of Reference for this report were adopted directly from the H_2S report with few changes. The Terms of Reference state that the focus of this scientific review is to be on the health effects of short-term exposure to SO_2.

The eligibility criteria for the selection of literature were also adopted directly from the H_2S report. The criteria were developed from the Terms of Reference. Only primary studies published in peer-reviewed publications were included in this review. Articles that were not primary scientific studies but were reviews themselves were not included, the primary goal of this review being an unbiased assessment of the scientific literature, not a re-reporting of previously published reviews. Studies reviewed included human clinical studies (clinical), animal toxicology studies (non-clinical), and population studies and case reports (epidemiology). 347 studies satisfied the final eligibility criteria for inclusion in this report, substantially more than for the H_2S report (45 studies) due in part to the inclusion of epidemiology studies.

Each study was critically assessed in terms of technical quality, including experimental design, conduct, and reporting. A level of confidence was assigned to each study based on the technical quality as judged by the reviewing team. The reviewing team consisted of seven members, all with scientific and/or epidemiologic backgrounds and extensive experience critically reviewing scientific literature. Each study was reviewed independently by three members of the reviewing team. The team members followed a pre-defined set of criteria for judging study quality. Of the 347 eligible studies reviewed, 184 (53%) were judged to be of low quality, 150 (43%) were of moderate quality, and only 15 (4%) were of high quality with no major weaknesses in study design or reporting.

The quality ranking of the studies was based on weaknesses or limitations identified by the reviewers. Some of the more common limitations identified included: too few study subjects, too few exposure concentrations (inability to determine dose-response relationship), failure to follow Good Laboratory Practice guidelines, failure to follow conventional testing protocols, critical information missing on experimental protocols, and unmeasured, poorly measured or unreported exposure concentrations and/or times. In drawing conclusions from this review, emphasis was placed on those studies ranked "high" or "moderate". These studies were judged to have the fewest limitations and therefore provided the strongest and most reliable evidence of association. For some health effects, few moderate or high quality studies were identified.

Results of animal and human studies were evaluated separately. No attempt was made to extrapolate from the animal testing evidence to human effects. It must also be emphasized that this report is a scientific review and as such the interpretations of the science do not represent policy or suggest public health implications.

The greatest number of studies, as well as the greatest number of high and moderate quality studies were those investigating respiratory effects as a result of SO_2 exposure. The strength-of-evidence for respiratory effects provided by these studies confirms that SO_2 exposure under certain conditions (exposure concentration, duration, and breathing method) can adversely affect the respiratory system. Human studies evaluating subjects with bronchopulmonary disease were included as well as those evaluating healthy subjects.

A. Evidence from Human Studies

Two types of studies were evaluated for evidence of effects on humans.

- Clinical studies involved controlled experiments on human volunteers.
- Epidemiology studies investigated short-term changes in health effects in populations with short-term changes in ambient concentration.

Both healthy subjects and those with respiratory illness (asthma or chronic obstructive pulmonary disease) were included in the studies.

Clinical studies covered a broad range of exposure durations. Therefore, the summaries of the findings are broken down by exposure time to facilitate comparison.

The weight of evidence for exposures up to 30 minutes suggests that healthy humans can experience exposures to SO_2 up to 10 ppm with transitory effects[1] on pulmonary function[2], even under challenging conditions involving hyperventilation, mouth-only exposure, and heavy exercise. Transitory effects may be observed at concentrations as low as 0.75 ppm.

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[1] Transitory effects: these effects were observed generally, but not always, for the duration of exposure with functioning returning to normal levels within minutes of hours of cessation of exposure.

[2] Pulmonary function or pulmonary effects: this refers primarily to spirometric changes (e.g. specific airways resistance, forced expiratory volume, etc.) that are measured in a clinical setting. In some cases, pulmonary effects may include clinical symptoms such as bronchoconstriction or throat irritation.

For exposures up to 30 minutes, asthmatics appear to demonstrate pulmonary effects at lower thresholds compared to healthy humans (0.1 ppm). However, even in this population subgroup the clinical effects are transient and may or may not require transient pharmacologic intervention.

The weight of evidence suggests that for single exposures up to 4 hours and repeated exposures between 3 days and 3 weeks, transitory pulmonary effects might occur for asthmatics at exposure concentrations between 0.5 and 1 ppm with exercise and for healthy humans between 0.75 and 25 ppm with exercise, with some evidence for a concentration-dependent response in healthy subjects.

Epidemiology studies were divided into two types based on presentation of exposure concentration. One set of studies calculated exposures as increases in ambient concentration above a baseline or average concentration. The other set of studies reported exposure as discrete concentrations, either as average concentrations or a concentration range.

A weight of evidence evaluation is difficult for the epidemiology studies. This is because the majority of the epidemiology studies (107 of 147) were ranked low quality. For those that ranked moderate quality, there were an equal number of studies that found insignificant or no associations between ambient SO_2 concentration and health outcomes as there were that reported an association.

Deriving causal associations from environmental epidemiologic studies is difficult for a number of reasons. No high quality epidemiology studies were identified. All of the epidemiology studies were subject to substantial limitations due to misclassification of either or both exposure and outcome. The majority of these studies are ecological in nature with outcomes determined on an individual level and exposure determined at a population level. The exposure data collected was generally for ambient levels. Since humans spend a large portion of their time indoors and travel through various microclimates during various activities, ambient levels will likely not provide a good measure of exposure at the individual level. Subsequently, the major weakness observed in these epidemiology studies is the potential for exposure misclassification as a result of the exposure assessment methods. Much of the exposure and outcome data used in these studies is retrospective and from public records, which increases the probability of misclassification due to inconsistent diagnosis of disease status or incorrect assessment of exposure. In addition, many confounding factors cannot be accounted for when using these types of data.

The epidemiology studies also present challenges for interpretation. The different exposure metrics used in the studies makes for difficulty in interpretation. For those studies looking at increases above a baseline, it should be noted that the baseline concentrations differ for each study. The time-averaging or time over which exposure was calculated is different between studies, making comparisons difficult. The populations used tended to be small and relatively undefined. For those studies that did report statistically significant results, the lower confidence intervals were often very close to one and there were few or no associations where the OR>2.

In addition, SO_2 is just one element in a mixture of pollutants found in "air pollution". It is difficult to isolate the effects of SO_2 from those of other single pollutants or combinations of pollutants. Because of these substantial limitations, the confidence in the results and conclusions from these epidemiology studies could not be judged to be higher than moderate and in most cases the confidence was judged to be low.

There is little reliable evidence in the peer-reviewed scientific literature that meets the terms of reference for this review of human health effects involving the eye, kidney and liver, or the cardiovascular, gastrointestinal, metabolic, immunological, reproductive, or nervous systems. It should be noted that SO_2 is generally considered an eye irritant. However, the conclusion in this report stems from the paucity of good-quality peer-reviewed scientific literature reporting specific effects on the eye. Much of the literature on reproductive effects on humans involves exposures longer than 30 days, which were not covered in this report, but will be covered in subsequent reports.

B. Evidence from animal studies

Much of the animal evidence for respiratory effects concentrates on the mechanisms of action of health effects from SO_2 exposure. Animal studies are also referred to as "**non-clinical**" studies.

As in the human clinical studies, the non-clinical animal studies covered a broad range of exposure durations. Therefore; the summaries of the findings are broken down by exposure time to facilitate comparison.

The concentrations in studies of animals exposed for up to 2 hours ranged between 0.5 ppm and 1000 ppm. For concentrations up to 100 ppm, effects reported were predominantly very mild respiratory effects and changes at the cellular or ciliary level. Above 100 ppm, greater pulmonary effects were in evidence, with indications of changes to the lung. There is evidence of increasing severity of effect with increasing concentration suggesting a possible dose response relationship.

In studies employing exposures between 2 and 24 hours, mild respiratory effects and delayed airway reactivity were reported with concentrations up to 40 ppm. Damage to the lungs was reported at concentrations of 800 ppm and 1225 ppm.

With exposures between 1 and 7 days, slight changes were observed in lung function and in response to virus challenges at concentrations of 0.1 ppm to 34.5 ppm. At the higher concentrations of 100 ppm and 600 ppm, changes to lung structure were reported.

Only five studies investigated exposures between 7 and 30 days. One study reported changes in response to virus challenges with exposures up to 0.1 ppm for 4 weeks. The other four studies reported changes in lung biochemistry and some decrease in pulmonary function at concentrations between 10 and 600 ppm.

Only a few animal studies looked at the effect of SO_2 exposure on the liver or

kidneys. However, there is some evidence of decreased levels of liver lipids and triglycerides and decreased enzyme activity in liver and kidney following continuous SO_2 exposure at 10 ppm for 15 days.
There is some evidence that exposure to SO_2 can affect the metabolic system, in particular lipid metabolism, at exposure times of several days. This effect seems to differ depending on which organ of the body is investigated.

There is some evidence from animal studies that SO_2 exposure both as an adult and prenatally can affect behaviour in adult mice subjected to challenging conditions. There is also some evidence that exposure to SO_2 can affect the lipid content of the brain. The outcomes of both these studies are of unknown clinical significance and the number of studies is limited, although the quality of the studies suggests the results are reliable. It has been established in several species that bronchial restriction upon SO_2 exposure is a reflex reaction; however, the mechanism of this reflex has not been conclusively determined.

In conclusion, there is limited animal evidence with respect to signs and symptoms, or effects on the eye, and reproductive, gastrointestinal, or cardiovascular systems found in the studies reviewed for this report.

TECHNICAL SUMMARY

Background

This report is the second comprehensive literature review commissioned by Alberta Health and Wellness in response to Recommendations 9 and 59 of the final report of the Provincial Advisory Committee on Public Safety and Sour Gas released in December 2000. These recommendations were concerned with the need to advance knowledge of the potential health effects of sour gas exposure. In addition, the Committee recommended that regulations reflect the current knowledge of sour gas and its components.

A comprehensive literature review on the health effects of short-term, low-level exposure to H_2S was released in December 2002 in response to Recommendation 9 (Cantox Environmental Inc., 2002). This review of the health effects of short-term exposure to low levels of SO_2 is in partial fulfillment of Recommendation 59. The H_2S report was prepared by Cantox Environmental Inc. of Calgary.

This current report is a critical review of health effects resulting from SO_2 exposure from published, peer-reviewed sources. Following the mandate of the Provincial Advisory Committee, the review focuses on the health effects of short-term exposures to SO_2. The purpose of this report is to develop a quantitative understanding of the current state of knowledge with respect to the dose-response relationship between exposure to SO_2 and health effects based on the weight of evidence in the peer-reviewed scientific literature.

An expert panel including members of the provincial government, industry, regional health authorities, and other interested stakeholders was convened to provide guidance for the H_2S review. This panel developed the terms of reference and criteria for evaluation of the literature, and provided ongoing guidance during the development of the H_2S review. While the expert panel did not meet regarding the SO_2 report, the Terms of Reference and criteria for evaluation were taken directly from the H_2S review with adaptations as required for SO_2. In addition, members of the panel were given the opportunity to comment and provide advice on the draft versions of this report. In many aspects, this report follows closely the structure and goals of the H_2S review.

The work began in June 2002 with the collection of the literature and was completed in October 2005 with the submission of this final report.

Terms of Reference

The terms of reference for this review are similar to those developed for the H_2S review. As such they are taken directly from the H_2S review, with adaptations as required to address SO_2. Reproductive and developmental effects of SO_2 exposure were not singled out in this report, although they were considered. The limitation to including these studies in this report is that most human studies investigating reproductive effects involve exposure times greater than 30 days. Reproductive effects will be more thoroughly considered in the subsequent report dealing with chronic exposure to SO_2.

The terms of reference, as adapted from the H_2S review for the SO_2 review, are:

- The review was to focus on the health effects following short-term exposure. The term "*short-term*" was to include exposures of both an acute and subacute variety, to capture exposures lasting a few hours to a few days. The subacute category was further defined to include exposures extending up to 30 days.

- The review was to focus on health effects *per se*. Although a formal definition of "*health effects*" was not adopted by the Expert Panel, the meaning was taken to be: *An undesirable or harmful effect on an organism with adverse consequences affecting survival, growth, development, performance, structure and/or function.*

  - The review was to be limited to information found in peer-reviewed scientific publications. Preference was to be given to English-language journals.

  - The review was to include all currently and readily available journal articles, with a strict need to avoid possible journal and/or sponsor bias.

  - The review was to focus on scientific studies involving exposures to SO_2 via inhalation to mimic the expected route of exposure of the general public. Studies involving other routes of exposures (e.g. oral, dermal, injection) were to be excluded from the review.

- The review was to focus on full-length, primary scientific investigations describing original work, rather than on review articles or abstracts.

- The review was to include information from clinical studies involving controlled exposures of human subjects in laboratory settings, non-clinical studies involving controlled exposures of test animals in the laboratory and "population" or epidemiology studies involving exposures following routine or accidental releases of SO_2 into the environment.

- The review was to include a critical assessment of the technical quality of each scientific paper based on consideration of experimental design, conduct and reporting. Judgment of quality was to be based on comparison against testing protocols recommended by leading scientific authorities.

Consistent with the H_2S review, this SO_2 review is strictly a scientific exercise. Only the technical criteria and the scientific meanings of the findings will be presented. Issues of public health implications or policy setting are beyond the scope of this review.

Studies investigating effects on hypersusceptible subjects (those with asthma, chronic obstructive pulmonary disease or hypersensitive airways) were included in addition to those investigating effects on healthy or normal subjects.

The focus of this work was the assessment of the body of scientific knowledge on the potential effects of acute exposure to SO_2 on humans. To that end, human and animal toxicology studies were included in this review. Effects on livestock were not considered.

Methods

As with the H_2S report, work on this report followed several defined stages. The first stage was the literature search. A preliminary search was initiated by Cantox Environmental Inc. The preliminary results formed part of the basis for a more extensive literature search by Alberta Health and Wellness. The purpose of the extensive literature search was to ensure all relevant studies were identified and ultimately included in the final report. Following the search and collection of the literature, a Reference Manager database was created. This served to assign an identifying number to each study for the purposes of the report. The inclusion of key words in the database allowed for the identification of studies focusing on various health effects. The studies were then reviewed following the quality criteria established for the H_2S report. A rating based on the quality criteria (low, moderate or high quality) was assigned to each study by the review team. The next step involved the interpretation of the studies, with the emphasis on studies rated moderate or high.

The search strategy followed for this report was similar to the H_2S search, but more refined with respect to the search terms used. The eligibility criteria for the SO_2 search were the same as for the H_2S search. One difference was the inclusion of epidemiology studies in the SO_2 report. Epidemiology studies investigating a link between short-term changes in exposure and short-term changes in health effects were included in this report. The inclusion of epidemiology studies substantially increased the number of studies included in the SO_2 report compared to the H_2S report. The eligibility criteria for the H_2S search were established by the members of the Expert Panel and reflect the Terms of Reference and were modified for the SO_2 search. The electronic search used the DIALOG Information Retrieval Service.

All studies were entered into a Reference Manager electronic database. In addition to bibliographic information, keywords and the abstracts of all the papers were included. The keywords corresponded to health effects that were to be highlighted in the final report. The unique identifying numbers assigned to the studies as well as the keywords in the database allowed for the easy location of studies pertaining to specific health effects.

347 studies met the eligibility criteria for inclusion in this report. These studies were judged according to pre-defined quality criteria established by the expert panel for the H_2S report. Each study was judged and ranked according to its technical quality as determined by the quality criteria.

A total of seven reviewers made up the reviewing panel for this report. Each paper was reviewed independently by three reviewers, with the goal being to reduce or eliminate reviewer bias. The reviewers represented a variety of scientific and/or epidemiological backgrounds and each had substantial experience critically reviewing scientific literature.

Other important notes:

- SO_2 is frequently used to induce bronchoconstriction in human and animal studies testing asthma medications. Studies of this type were included if the effect of SO_2 alone could be determined in the study with no interaction with the medication being tested.

- There was no upper limit set for the exposure concentrations used in the studies included in this review in order to provide a complete overview of the scientific literature, and to provide a full picture of the potential health effects of SO_2 exposure.

- Some non-clinical studies report exposure close to chronic exposure (longer than 30 days). These studies were included in this review when effects were reported at time periods shorter than 30 days (i.e. shorter than the full exposure time in the study).

- Some studies report concentrations in units other than ppm or ppb. All units have been converted to ppm or ppb for consistency and to facilitate comparison. The equation used for the conversion was:

  $\text{ppm} = \text{mg/m}^3 \times 24.45/\text{mol. wt.}$

  Where:

  24.45 is the volume of 1 mole of air at 25°C and 1 atmosphere; and
  mol. wt. is the molecular weight of $SO_2 = 64.06$

Findings

The 347 studies included in the review were assessed based on their technical quality. Only 15 studies (4%) were judged to be of "high" quality with no major flaws in study design or reporting. 149 studies (43%) were found to be of "moderate" quality with some weaknesses or limitations in either study design or reporting. 183 studies (53%) were judged to have major limitations in study design, conduct, or reporting and were classified as being of poor or "low" quality. Common limitations in those studies rated "moderate" or "low" were very similar to the limitations found in the H_2S literature. Studies covered a broad range of exposure durations. Therefore, the summaries of the findings are broken down by exposure time to facilitate comparison.

A limitation unique to the epidemiology studies is inaccurate or inadequate exposure assessment. This is a common problem in environmental epidemiology. These studies generally compared ambient exposures as measured at monitoring stations with various health effects based on the assumption that ambient monitoring is an accurate description of the exposure of each individual to the contaminants in question. Given that human beings spend a large portion of time indoors, particularly in the Northern Hemisphere, and travel through several microclimates during the course of their various activities, ambient concentrations likely do not accurately reflect SO_2 levels

experienced by individuals. In addition, SO_2 is only one of many compounds in the ambient air. Several studies attempted to account for the influence of other compounds. However, this is a limitation that must be taken into account. Epidemiology studies are vulnerable to exposure misclassification, which consequently weakens confidence in the reported results.
Outcome misclassification is a potential limitation of epidemiology studies using population data for outcome determination. Population data from hospital or medical records may be incomplete and are subject to misclassification or information bias with regards to diagnosis of the disease or cause of death. This is particularly the case for many respiratory diseases that may not have standard case definitions. Epidemiology studies cannot demonstrate causation, they can only demonstrate association. In other words, epidemiology studies, particularly of the type seen in this review, are hypothesis generating, not hypothesis testing. Epidemiology studies also rely heavily on statistical methods and statistical software to "smooth" the data and identify association. This can result in problems if the software is faulty, for example, the S-Plus issues identified that resulted in the reanalysis of several studies.

Several weaknesses common to many human clinical and animal toxicology (non-clinical) studies included:

- ❖ Use of a single exposure concentration, precluding any attempt at determining a dose-response relationship, an important criterion for a causal association.
- ❖ Use of a single sex, precluding generalization to a larger population.
- ❖ Use of too few test subjects, making it difficult to interpret the significance of test results.
- ❖ Lack of routine measures of toxicity, such as signs and symptoms, body weights, and pathology (especially for non-clinical studies). Failure to report these indices makes it difficult to interpret the clinical significance of other observed effects.
- ❖ Failure to follow conventional testing protocols and Good Laboratory Practice guidelines.
- ❖ Failure to adequately describe exposure conditions, such as concentrations, times, and method of exposure, acclimation protocols (mouthpiece, chamber, etc.).
- ❖ Failure to adequately describe characteristics of the test subjects (sex, age, weight, pre-trial health)

Several of the studies, particularly those reviewed in the nervous system, immunological, and respiratory-biochemical effects sections, focussed on the mechanisms of action of SO_2 toxicity. However, the effects were seen at subclinical levels and subsequent clinical repercussions are unclear.

The studies were grouped on a system-by-system basis, following the H_2S report.

Mortality

Clinical Studies

No clinical studies on humans used mortality as a health endpoint, for ethical reasons.

Non-Clinical Studies

Of 7 non-clinical studies investigating mortality of animals, 4 of those were of high or moderate quality. Of those four studies, one high quality study found an increase in mortality rate and decreased survival time of mice with bacterial infections after exposure to 10 ppm SO_2 for one week or longer. However, a moderate quality study reported no change in mortality from bacterial infection in mice exposed to 0.95 ppm for two hours. Two studies observed increases in mortality rates in mice or chickens with increasing exposure time and SO_2 concentration. In mice the SO_2 concentrations ranged from 900 to 1900 ppm for times of 10 to 640 minutes. For chickens, the concentrations ranged from 1 to 5000 ppm for 60 minutes with deaths occurring above 1000 ppm. One low quality study attempted to determine the LC_{50} in mice at various concentrations and times. However, this study had many limitations, including failure to follow Good Laboratory Practice guidelines.

Epidemiology Studies

Fifty epidemiology studies and case reports were identified that investigated an association between SO_2 exposure and mortality. Only 13 of those studies were evaluated to be of moderate quality. The majority of the epidemiology studies are ecological in nature with outcomes determined on an individual level and exposure determined at a population level. The exposure data collected is generally of ambient levels. Since humans spend a large portion of their time indoors and travel through various microclimates during their daily activities, ambient levels will likely not be a good measure of exposure at the individual level. These studies are subject to the "ecological fallacy" where outcomes and exposures are erroneously ascribed to individuals when only group or population data are available. With the ecological fallacy, an association might be observed on a population level between an outcome and an exposure. However, because of the lack of individual data, we cannot be sure that the individuals who display the outcome are the same individuals who experienced the exposure of interest. Another major weakness observed in these epidemiology studies is the potential for exposure misclassification as a result of the exposure assessment methods. Much of the exposure and outcome data used in these studies is retrospective and from public records, which increases the probability of misclassification bias. Many confounding factors cannot be accounted for when using these types of data. In addition, SO_2 is just one element in a mixture of pollutants found in "air pollution". It is difficult to isolate the effects of SO_2 from those of other single pollutants or combinations of pollutants. Because of these substantial limitations, the confidence in the results and conclusions from these epidemiology studies could not be judged to be higher than moderate and in most cases the confidence was judged to be low.

The epidemiology studies reported exposure metrics as either incremental increases in concentration or as absolute concentrations. It is important to keep this distinction in mind when interpreting the results of the studies. With incremental increases in concentration, it is important to be aware that the relative incremental increase occurs in relation to a certain background or average level. For example, when a study reports health effects per 38 ppb increase in ambient concentration, we must understand whether the baseline concentration is 400 ppb or 40 ppb. In other words, are the studies investigating concentrations of 400, 438, 476, and so on or concentrations of 40, 78, 116, etc. This is also important when studies report relative descriptors for exposure concentration increases such as a "doubling" or a "four-fold increase". A doubling of concentration from 4 ppb to 8 ppb will have different implications for human health than a doubling from 400 ppb to 800 ppb. Knowledge of the absolute concentrations will aid in the interpretation of the study results.

Keeping these limitations in mind, general conclusions can be extracted from the moderate quality studies. Several European studies observed an association between incremental increases in SO_2 and daily all-cause mortality for a wide range of baseline concentrations. These studies were part of the APHEA (Air Pollution and Health, a European Approach) project. However, not all of the APHEA studies observed an association between an incremental SO_2 increase and mortality. There was substantial variation in results among the APHEA participant cities. Other studies observed small and sometimes significant associations between a variety of incremental and absolute concentrations and all-cause or cause-specific mortality.

Respiratory System

The majority of studies investigated respiratory health effects related to SO_2 exposure. In addition to these summary paragraphs, please refer to Tables 1 to 9 and Figures 1 to 7.

Clinical Studies

Of the 96 clinical studies investigating respiratory effects, 73 (76%) were ranked high or moderate. Clinical studies covered a broad range of exposure durations. Therefore, the summaries of the findings are broken down by exposure time to facilitate comparison.

There were no high quality studies looking at healthy humans in the time range of 1 to 10 minutes of exposure. There were, however, a number of moderate quality studies. Pulmonary effects[3] in healthy humans starting at 0.75 ppm and up to 15 ppm were observed in clinical studies. These studies involved direct exposure to SO_2 with hyperventilation and/or exercise. There is some evidence that pulmonary effects are greater when exposure is through a mouthpiece (orally) than through the nose. Dryness, irritation and burning of the throat were observed at 3, 15, and 28 ppm in two moderate quality studies.

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[3] Pulmonary function, pulmonary effects, or respiratory function: these phrases refer primarily to spirometric changes (e.g. specific airways resistance, forced expiratory volume, etc.) that are measured in a clinical setting. In some cases, pulmonary effects may include clinical symptoms such as bronchoconstriction or throat irritation.

Only one study of 27 investigating exposure of asthmatics for 1 to 10 minutes was rated high quality. This study noted a concentration-dependent change in respiratory function in asthmatics between 0.5 and 1 ppm with exposure to SO_2 during light to heavy exercise. The moderate quality studies also involved direct SO_2 exposure, usually with exercise and/or hyperventilation. Small but significant pulmonary effects were observed in asthmatics at concentrations ranging between 0.1 ppm to 10 ppm. These effects were transitory[4] and pulmonary function returned to normal after exposure ceased. Again, the literature suggests there is evidence that mouth breathing or oral exposure results in greater health effects than nasal exposure.

Pulmonary effects were observed at concentrations as low as 1 ppm at exposures times between 11 and 30 minutes. Again, these effects were transitory. Three studies investigated the effects on cells from the respiratory system after exposure to concentrations between 2.5 and 8 ppm. Some effect was observed on these cells.

Only one study was assessed as high quality for exposures between 11 and 30 minutes. Pulmonary function effects were observed in asthmatics upon exposure to 0.5 ppm SO_2 with moderate exercise.

Other studies suggest pulmonary effects with exercise at concentrations between 0.1 ppm and 1 ppm.

Few studies investigated exposures in asthmatics longer than 30 minutes. Those that did reported transitory pulmonary function effects at exposure levels of 0.50 to 1 ppm. The studies investigating healthy subjects at these longer time ranges investigated concentrations between 0.4 and 25 ppm. A concentration-dependent response in discomfort was reported between 1 and 25 ppm. Transitory effects on pulmonary function and nasal mucous flow were reported up to 5 ppm at these longer time ranges.

The weight of evidence for exposures up to 30 minutes suggests that healthy humans can experience exposures to SO_2 up to 10 ppm with transitory effects on pulmonary function, even under challenging conditions involving hyperventilation, mouth-only exposure, and heavy exercise. Transitory effects may be observed at concentrations as low as 0.75 ppm.
For exposures up to 30 minutes, asthmatics appear to demonstrate pulmonary effects at lower thresholds (0.1 ppm), although even in this population subgroup the clinical effects are transient and may or may not require transient pharmacologic intervention.

The weight of evidence for single exposures up to 4 hours and repeated exposures between 3 days and 3 weeks suggests that transitory pulmonary effects might be expected for asthmatics at exposure concentrations between 0.5 and 1 ppm and for healthy humans between 0.75 and 25 ppm, with some evidence for a concentration-dependent response in healthy subjects.

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[4] Transitory effects: these effects were observed generally, but not always, for the duration of exposure with functioning returning to normal levels within minutes of hours of cessation of exposure.

Of the 93 non-clinical studies investigating respiratory effects, 39 (42%) were ranked high or moderate. These studies looked at a variety of species and health outcomes. In addition there was substantial variation in the concentrations and exposure times investigated. Exposures included single exposures of up to a few hours to several days as well as multiple exposures of a few hours per day for up to 30 days.

The concentrations in studies of animals exposed for up to 2 hours ranged between 0.5 ppm and 1000 ppm. For concentrations up to 100 ppm, effects reported were predominantly very mild respiratory effects and changes at the cellular or ciliary level. Above 100 ppm, greater pulmonary effects were present, with indications of changes to the lung. There is evidence of increasing severity of effect with increasing concentration.

In studies with exposures between 2 and 24 hours, mild respiratory effects and delayed airway reactivity was reported with concentrations up to 40 ppm. Damage to the lungs was reported at concentrations of 800 ppm and 1225 ppm.

At exposures between 1 and 7 days, slight changes were observed in lung function and in response to virus challenges at concentrations between 0.1 ppm and 34.5 ppm. At the higher concentrations of 100 ppm and 600 ppm, changes to lung structure were reported.

Only five studies investigated exposures between 7 and 30 days. One study reported changes in response to virus challenges with exposures up to 0.1 ppm for 4 weeks. The other four studies reported changes in lung biochemistry and some decrease in pulmonary function at concentrations between 10 and 600 ppm.

Of the epidemiology studies and case reports investigating respiratory effects, less than half were ranked moderate. There were no high quality epidemiology studies.
As in the mortality section, epidemiology studies employed two types of metric for exposure concentration. One set of studies calculated exposures as increases in ambient concentration above a baseline or average concentration (incremental). These studies report results as an increase in outcomes (e.g. hospital admissions for asthma) per increase in ambient concentration. For example, a study might report results as a 1.6% increase in hospital admissions for every 3.5 ppm increase in ambient SO_2 concentration. Another set of studies reported exposure as discrete or absolute concentrations, either as average concentrations or a concentration range. These studies might report results as, for example, 7% more admissions during periods of higher pollution.

A weight of evidence evaluation is difficult for the epidemiology studies as the majority of the epidemiology studies were ranked low quality. For the moderate quality studies reporting both types of exposure metric, there was an equal number of studies that found insignificant or no associations between ambient SO_2 concentration and health outcomes as there were that did report an association.

The limitations of the respiratory epidemiology studies are similar to those outlined in the mortality section. Exposure misclassification is a major limitation of these studies. An additional limitation involves the classification of outcome. In several cases, the respiratory diseases investigated, particularly COPD and asthma, did not have clear case definitions for the purposes of the study, which could lead to inaccurate or inconsistent diagnoses of the health outcomes. The issue of incremental and absolute exposure metrics has been discussed. In addition, for those studies looking at increases above a baseline, it should be noted that the baseline concentrations differ for each study. In addition, the time-averaging or time over which exposure was calculated is different between studies, making comparisons difficult. The populations used tended to be small and relatively undefined. Most of the studies endeavored to find correlations between ambient levels of SO_2 and rates of health outcomes. Very few calculated relative risks or similar epidemiological statistics. For those studies that did report statistically significant results, the lower confidence intervals were often very close to one and there were no associations with an OR>2.

Signs and Symptoms

Clinical Studies

Several clinical studies reported signs and symptoms as observations concurrent to investigation of other effects. Healthy subjects reported nose and throat irritation, taste and odour complaints, and discomfort during single exposures (15 and 28 ppm for 10 minutes or less than 1 ppm for 40 minutes) as well as during multiple exposures (1 ppm for 4 hours/day, 3 days/week for 3 weeks and 1 to 25 ppm for 6 hours/day for 3 consecutive days). Some coughing was observed in the healthy subjects during forced mouth breathing. Asthmatic subjects reported chest tightness, shortness of breath, wheezing, asthma symptoms, dyspnea and cough during single exposure both with and without exercise. Concentrations ranged from 0.5 to 1 ppm and lasted between 3 minutes and 3 hours. No symptoms were reported in asthmatics after exposure to 0.5 ppm for one hour, subjects with COPD exposed to 0.8 ppm for one hour, or healthy subjects exposed to 0.15 ppm for 2 hours.

Non-Clinical Studies

Few non-clinical studies reported irritative symptoms as a result of SO_2 exposure. Itching, preening, somnolence, and eye-irritation were observed in guinea pigs exposed to 10 ppm for 1 hour/day for 21 days. Depressed feed and water intake and decreased body weight and oxygen consumption were observed in male mice exposed to 40 ppm for 4 to 11 days. Recovery of body weight and oxygen consumption began immediately after cessation of exposure.

Epidemiology Studies

Shortcomings in epidemiology studies and case-reports have been detailed in the mortality and respiratory summaries of this report. The same limitations apply to the few moderate epidemiology studies and case-reports reporting general signs and symptoms. Responders in a telephone survey during elevated air pollution events with ambient SO_2 levels up to 0.15 ppm reported increased eye and throat irritation, chest discomfort, shortness of breath, and restricted activity. Two miners exposed to very high concentrations in a mine explosion reported reduced exercise tolerance two years after the accident. No association was reported between ambient SO_2 concentrations up to 3.3 ppm and hospital admissions for asthma, wheeze, or shortness of breath.

Cardiovascular System

There were few studies that investigated the effects of SO_2 on the cardiovascular system including only one moderate quality clinical study. This study reported weak evidence of a difference in electrocardiogram readings after exposure to 0.2 ppm for 1 hour.

Non-Clinical Studies

A high quality non-clinical study reported an increase in the heart rate of chickens with exposure to 5000 ppm for 1 hour, but no effect on heart rate or blood pressure at exposure to 100 ppm for 1 hour. Two moderate quality studies also investigated effects on heart rate and blood pressure. Rats exhibited decreased heart rate after two tidal breaths of 5000 ppm. Geese exhibited increased blood pressure and heart rate with 1 to 3 minutes of exposure to 100 to 400 ppm.

Multiple exposure designs identified decreased glutathione in the hearts of rats (5 to 100 ppm for 5 hours a day for 28 days) and an increase in cholesterol, total lipids, and phospholipids in guinea pig hearts (10 ppm for 1 hour/day for 30 days). The clinical significant of these results is unclear.

Epidemiology Studies

In the lone moderate epidemiology study investigating this system, a small but significant association was reported between daily admission for cardiac disease in London England and Hong Kong and an incremental daily 4 ppb increase (baseline concentrations: 6.8 ± 4.7 ppb) in ambient SO_2 concentrations. The limitations previously identified for epidemiology studies apply to this study. In particular, exposure assessment was a major limitation and the study was rated "moderate-to-low".

Eye

SO_2 is generally acknowledged to have irritant effects on the eye. However, very few studies of the peer-reviewed, scientific studies fitting the terms of reference for this report reported eye effects and none of the studies focussed specifically on investigating eye effects. Some studies with a focus on other health endpoints reported eye effects as a peripheral observation.

Clinical Studies

Of the three clinical studies reporting eye effects, one was rated of high quality while the other two were low quality. The high quality study reported no adverse effects on the eye with exposure to 1 ppm for 4 hours/day, 3 days/week for 3 weeks.

Non-Clinical Studies

The single non-clinical study reporting eye effects was ranked high quality. Eye effects were not a major focus of this study. However, the study reports that exposure of guinea pigs to 10 ppm for 1 hour/day for 12 days leads to signs of eye irritation.

Epidemiology Studies

Only one of four epidemiology studies mentioning eye effects was ranked moderate quality. This study reported that increases in eye irritation were observed during elevated pollution events with ambient levels up to 0.15 ppm.

Gastrointestinal System
No studies clinical or epidemiology were found that investigated or reported effects to the gastrointestinal system as a result of acute SO_2 exposure.

Non-Clinical Studies
One moderate non-clinical study suggested that inhalation of SO_2 (8.4±0.8, 21±1, and 43±3 ppm) increased levels of lipid peroxidation in stomachs and intestines of male and female mice. These results suggest a toxicological role of SO_2 inhalation on these organs in mice. Confidence intervals were not reported, but Good Laboratory Practice guidelines were generally followed.

General Biochemical Effects

Clinical Studies
Two moderate quality clinical studies were identified. In one study, no association was observed between plasma antioxidant nutrient concentrations and sensitivity to inhaled SO_2. In the other study, a dose-dependent stimulus of reactive oxygen intermediate (ROI) was reported with exposure to concentrations between 0.3 and 1.5 ppm for 30 to 60 minutes. However, it is unclear how much ROI stimulation is required to induce clinically relevant pulmonary fibrosis.

Non-Clinical Studies
Two high quality non-clinical studies were identified. One investigated the effects of SO_2 exposure on serum lipids and lipoproteins and glucose metabolism in diabetic and normal rats. With continuous exposures of 5 and 10 ppm for 15 days, increases in plasma triglycerides and decreases in plasma HDL cholesterol were reported in the healthy rats. Increases in plasma triglycerides and increases in plasma HDL cholesterol were reported in the diabetic rats. The other high quality study investigated responses of chickens to high levels of SO_2 (5000 ppm for 1 hour). Decreased blood pH and increased blood CO_2 were observed. Moderate quality studies reported increased sulfhemoglobin values, and lower whole blood and packed cell viscosity, but no differences in plasma viscosity (0.87 ppm for 24 hours in rats), and a time-dependent decrease in plasma thyroxine levels in mice exposed to 40 ppm for 12 to 24 hours. One study reported no differences in blood variables or hemoglobin affinity in rats exposed to 2 ppm continuously for 1 to 49 days.

No epidemiology studies were identified that fit the criteria for inclusion.

Immunological System

Clinical Studies
Several clinical studies investigated the mechanisms of action of SO_2 on immunological system functions. Increased alveolar macrophage activity was reported in subjects exposed to 4 and 8 ppm for 20 minutes. One study induced rhinovirus infection in a SO_2-exposed group (5 ppm for 4 hours) and a control group. The number of subjects who developed colds was not different between the two groups. However, the SO_2-exposed group experienced a decrease in nasal mucus flow rate, fewer symptoms, and less but more persistent

virus shedding. It has been suggested that mechanisms of asthmatic sensitivity may be associated with a wild-type allele of the TNF-alpha promoter polymorphism or may involve mast cell degranulation.

Non-Clinical Studies

Increased mortality and decreased survival time was observed in a group of female mice with respiratory infection exposed to 10 ppm for up to 3 weeks compared to non-exposed controls. Mice exposed to 0.03 to 0.1 ppm and an influenza virus developed antibodies to the virus more rapidly than mice exposed to the virus alone. The study authors postulate from this that SO_2 alters nasal mucus membranes thereby decreasing a defensive barrier to disease and resulting in increased severity of influenza infection. However, another study reported that exposure to 6 ppm for 7 days resulted in partial inhibition of influenza virus growth in the nasal epithelium and no propagation in the lungs. Studies on guinea pigs suggested that exposure to low levels of SO_2 (1 ppm) might enhance the development of ovalbumin-induced asthmatic reactions and reported a significant increase in ovalbumin-specific antibodies in serum and bronchoalveolar fluid with exposure to 0.1 to 16.6 ppm for 8 hours/day for 5 days. A study on mice exposed to 250 ppm for 3 hours reported an increased uptake of iron in airway epithelium. The clinical significance of many of these studies is unclear and not discussed in the studies themselves.

Epidemiology studies

One moderate quality epidemiology study reported that children with bronchial responsiveness and high serum concentrations of total IgE were particularly susceptible to air pollution, but not SO_2 specifically.

Kidney and Liver

No human clinical or epidemiology studies investigating or reporting liver or kidney effects and fitting the criteria were identified for this review. There were, however, several animal studies.

Non-Clinical Studies

Increases in liver weight and triglycerides in the livers of healthy rats exposed to 10 ppm continuously for 15 days were observed in a high quality study. The same study reported decreased liver weight and a dose-dependent decrease in liver triglycerides in diabetic rats after exposure to 5 or 10 ppm continuously for 15 days. Depletion of phospholipids, cholesterol, cholesterol/phospholipid ratios and lipid peroxidation in guinea pig livers was reported after exposure to 10 ppm for 1 hour/day for 30 days. Glutathione reductase activity was decreased in rat livers at 5 pm for 5 hours/day for 7 to 28 days. In addition, glutathione levels in the liver and kidney were reduced at concentrations between 5 and 100 ppm for the same exposure protocol.

Metabolic System

No human clinical or epidemiology studies were identified that investigated this health outcome and fit the criteria.

Non-Clinical Studies

Continuous exposure of mice to 40 ppm for 4 to 11 days was reported to depress metabolism as measured by oxygen consumption. Decreased enzyme activity was observed in mice (20 ppm for 6 hours/day for 7 days) and rats (5 to 10 ppm for 5 hours/day for 7 to 28 days). Clinical significance of these observations was not discussed and is unclear.

Changes in lipid metabolism were reported in rats (continuous exposure to 5 and 10 ppm for 15 days) and guinea pigs (20 ppm for 1 hour/day for 30 days).

Nervous System

No human clinical or epidemiology studies were identified as fitting the criteria.

Non-Clinical Studies

Behavioural changes in rearing, social interactions, grooming, digging and chamber-crossing were reported in male and female mice exposed to 5, 12, and 30 ppm of near continuous exposure for 24 days. Male mice exposed to 5, 12, and 30 ppm prenatally exhibited changed aggressive behaviour in adulthood when subjected to an aggressive encounter with an unexposed mouse of the same age, body weight and isolation condition.

Changes in the lipid content of guinea pig and rat brains were reported for exposure to 10 ppm for 1 hour/day for 21 days and 30 days, respectively.

Several studies investigated the effect of SO_2 exposure on respiratory reflex mechanisms. Theses studies concur that the bronchoconstrictive response is reflexive, but the mechanism of the reflex has not been conclusively identified.

Olfactory System

Unlike for H_2S, there are no studies investigating the effect of SO_2 exposure on the sense of smell. Studies concerned with the effects of SO_2 on the nasal passages are described in the section on the respiratory system.

Reproductive System

No clinical studies or moderate or high quality epidemiology studies were identified for this health outcome.

Non-Clinical Studies

No significant teratological or embryotoxicological effects were reported in studies on mice exposed to up to 250 ppm during gestation.

No changes in reproductive performance or neurobehavioral development were reported in male and female mice exposed to up to 30 ppm during gestation. Some social or agonistic behavioural changes were reported during an aggressive encounter in adult male mice that had been exposed to up to 30 ppm during gestation.

Conclusions

The majority of the evidence from the scientific literature reviewed here refers to effects on the respiratory system. There is limited evidence, primarily from animal studies, of effects to other body systems.

Evidence from Human Studies

Both healthy subjects and those with respiratory illness (asthma or chronic obstructive pulmonary disease) were included in this review. The most common effects reported in healthy subjects upon acute exposure to SO_2 include increased airway resistance and bronchoconstriction, decreased maximum expiratory flow, and decreased pulmonary function. Some subjects reported dryness and irritation of the throat, general respiratory discomfort, and unpleasant taste and odours. Effects reported in asthmatic subjects were similar, but also included increases in asthma symptoms, wheezing, chest tightness, and dyspnea. The weight of evidence suggests that subjects with respiratory illness are more susceptible to respiratory health effects from SO_2 exposure.

Other factors contributing to SO_2-induced effects were examined in these studies. Exercise seems to exacerbate the response to SO_2 in both healthy and asthmatic subjects. Cold and/or dry air also exacerbates the asthmatic response. In addition, the method of exposure affects the response, with forced mouth breathing eliciting a greater response than nasal or oronasal breathing.

Clinical studies covered a broad range of exposure durations. Therefore, the summaries of the findings are broken down by exposure time to facilitate comparison.

The weight of evidence for exposures up to 30 minutes suggests that healthy humans can experience exposures to SO_2 up to 10 ppm with transitory effects on pulmonary function, even under challenging conditions involving hyperventilation, mouth-only exposure, and heavy exercise. Transitory effects may be observed at concentrations as low as 0.75 ppm.

For exposures up to 30 minutes, asthmatics appear to demonstrate pulmonary effects at lower thresholds (0.1 ppm), although even in this population subgroup the clinical effects are transient and may or may not require intermittent pharmacologic intervention. The weight of evidence for single exposures up to 4 hours and repeated exposures between 3 days and 3 weeks suggests that transitory pulmonary effects might be expected for asthmatics at exposure concentrations between 0.5 and 1 ppm and for healthy humans between 0.75 and 25 ppm, with some evidence for a concentration-dependent response in healthy subjects.

No high quality epidemiology studies or case reports were identified. A weight of evidence evaluation is difficult for the epidemiology studies as the majority of these studies were ranked low quality. For the moderate quality studies reporting both types of exposure metric, there was an equal number of studies that found insignificant or no associations between ambient SO_2 concentration and health outcomes as there were that reported an association. These studies were subject to substantial limitations due to misclassification of both exposure and outcome as well as other limitations outlined previously. Because of these substantial limitations, the confidence in the results and conclusions from these epidemiology studies could not be judged to be higher than moderate and in most cases the confidence was judged to be low.

Associations, when reported, were generally weak. Associations were reported for decreased pulmonary function, and hospital admissions for asthma and other respiratory diseases. Reported symptoms included throat irritation, chest discomfort, restricted activity, shortness of breath, cough, dyspnea, and lower baseline function. Weak associations were reported in epidemiology studies for various mortality causes. However, the body of epidemiological evidence for mortality contains much variability and few studies in which we can have confidence, mainly due to the limitations discussed previously.

There is little reliable evidence in the peer-reviewed scientific literature fitting the terms of reference for this report of human health effects involving the eye, kidney and liver, or the cardiovascular, gastrointestinal, metabolic, immunological, reproductive, or nervous systems.

Evidence from animal studies
Much of the animal evidence for respiratory effects concentrates on the mechanisms of action of health effects from SO_2 exposure. The clinical significance of much of the animal evidence is unclear and was not discussed in the studies themselves. Studies on respiratory effects were well represented. Reported respiratory effects included increased bronchoconstriction and specific airway resistance and decreased ciliary activity.
Non-clinical studies also covered a broad range of exposure durations. Therefore, the summaries of the findings are broken down by exposure time to facilitate comparison.
The concentrations in respiratory studies of animals exposed for up to 2 hours ranged between 0.5 ppm and 1000 ppm. For concentrations up to 100 ppm, effects reported were predominantly very mild respiratory effects and changes at the cellular or ciliary level. Above 100 ppm, pulmonary effects were more pronounced, with indications of changes to the lung. There is evidence of increasing severity of effect with increasing concentration.

In studies with exposures between 2 and 24 hours, mild respiratory effects and delayed airway reactivity were reported with concentrations up to 40 ppm. Damage to the lungs was reported at concentrations of 800 ppm and 1225 ppm.

At exposures between 1 and 7 days, slight changes in lung function and in response to virus challenges were observed at concentrations between 0.1 ppm and 34.5 ppm. At the higher concentrations of 100 ppm and 600 ppm, changes to lung structure were reported.

Few respiratory studies investigated exposures between 7 and 30 days. One study reported that changes were observed in response to virus challenges with exposures up to 0.1 ppm. The other studies reported changes in lung biochemistry and some decrease in pulmonary function at concentrations between 10 and 600 ppm.

Only a few animal studies looked at the effect of SO_2 exposure on the liver or kidneys. However, there is some evidence of decreased levels of liver lipids and triglycerides and decreased enzyme activity in liver and kidney following continuous SO_2 exposure at 10 ppm for 15 days.
There is some evidence that exposure to SO_2 can affect the metabolic system, in particular lipid metabolism, at exposure times of several days. This effect seems to differ depending on which organ of the body is investigated.
There is some evidence from animal studies that SO_2 exposure both as an adult and prenatally can affect behaviour in adult mice subjected to challenging conditions. There is also some evidence that exposure to SO_2 can affect the lipid content of the brain. The outcomes of both these studies are of unknown clinical significance and, the number of studies is limited, although the quality of the studies suggests the results are

reliable. It has been established in several species that bronchial restriction upon SO_2 exposure is a reflex reaction; however, the mechanism of this reflex has not been conclusively determined.

There is limited animal evidence for signs and symptoms, or effects on the eye, and reproductive, gastrointestinal, or cardiovascular systems found in the animal studies reviewed for this report.

I. BACKGROUND

This report is the second comprehensive literature review commissioned by Alberta Health and Wellness in response to Recommendations 9 and 59 of the final report of the Provincial Advisory Committee on Public Safety and Sour Gas released in December 2000 (ref). These recommendations were concerned with the need to advance knowledge of the potential health effects of sour gas exposure. In addition, the Committee recommended that regulations reflect the current knowledge of sour gas and its components. The two recommendations leading directly to this report and the recently released H_2S report (Cantox Environmental Inc., 2002) are:

Recommendation 9
The EUB work with Alberta Health and Wellness, regional health authorities, Alberta Environment, Alberta Human Resources, industry and other stakeholders to ensure that comprehensive health effects information (qualitative and quantitative) is developed, as soon as practical due to its urgency.

Recommendation 59
The EUB work with Alberta Health and Wellness, regional health authorities and other stakeholders to develop clear requirements and evacuation criteria to address the hazard of SO_2 *as a result of ignition.*

The comprehensive literature review on the health effects of acute exposure to H_2S was released in December 2002 in response to Recommendation 9 (Cantox Environmental Inc., 2002), hereafter referred to as "the H_2S report". The H_2S report was prepared by Cantox Environmental Inc. of Calgary. The present review of the health effects of acute exposure to low levels of SO_2 is in partial fulfillment of Recommendation 59.

This current report is a critical review of the scientific information on health effects from SO_2 exposure currently available from published, peer-reviewed sources. The purpose of this report is to develop a quantitative understanding of the current state of knowledge with respect to the dose-response relationship between exposure to SO_2 and health effects based on the weight of evidence in the peer-reviewed scientific literature. Following the mandate of the Provincial Advisory Committee, the review focuses on the health effects of acute or short-term exposures to SO_2.

The guidance of the expert panel commissioned for the H_2S report was also applied to this SO_2 report although the expert panel was not specifically re-convened during the initial stages of the SO_2 work. The SO_2 report was modeled on the H_2S report as closely as possible with respect to scope, terms of reference, and criteria for evaluation of the literature. This expert panel for the H_2S report included members of the provincial government, industry, regional health authorities, and other interested stakeholders. The expert panel was asked to comment on draft versions of the report and their input was included in the final version.

The work began in June 2002 with the search and collection of the references and was completed in September 2004 with the submission of this final report.

II. INTRODUCTION

This review is the second comprehensive literature review commissioned by AH&W on the health effects of acute exposure to sour gas components. The first report was the H_2S review that was completed in July 2002 by Cantox Environmental Inc. of Calgary. Work on this SO_2 review began in June 2002. The scope and nature of the H_2S review was defined by an Expert Panel drawn from Alberta Environment, regional health authorities, industry and other interested stakeholders. The terms of reference and scope defined by the Expert Panel in the H_2S review are applied to this SO_2 review.

The structure of the SO_2 report closely follows that of the H_2S report. Selected definitions that are important to ensuring full understanding of the terms of this report were developed in the H_2S report in discussions with the Expert Panel and are adopted here (taken directly from the H_2S report):

short-term, *adj*. **1**. extending over a few hours of a few days. **2**. encompassing acute and subacute events lasting up to 30 days.

health effect, *n*. an undesirable or harmful effect on an organism with adverse consequences affecting survival, growth, development, performance, structure and/or function.

The H_2S report was primarily concerned with exposures below 100 ppm due to the fairly well established results of exposure to high concentrations of H_2S, in particular "H_2S knockdown". However, the effects of exposure to higher levels of SO_2 are not as well characterized. Therefore, no upper SO_2 exposure concentration cut-off was established and studies evaluating higher concentrations of SO_2 are included. These constitute mainly the non-clinical animal trials.

The guidance of the expert advisory panel commissioned for the H_2S report was incorporated into this SO_2 report. The members of the expert panel were:

Dr. Randy Angle
(Alberta Environment)

Mr. Justin Balko
(Alberta Health and Wellness)

Dr. Nicholas Bayliss
(Alberta Health and Wellness)

Dr. Donald Davies ("consultant")
(Cantox Environmental Inc.)

Dr. Stephan Gabos
(Alberta Health and Wellness)

Mr. Geoffrey Granville
(Shell Canada Ltd.)

Mr. Alex MacKenzie
(Alberta Health and Wellness)

Dr. Ingrid Vicas
(Calgary Health Region – Alberta Poison Centre)

Funding for the review team was provided by Alberta Environment.

III. TERMS OF REFERENCE

The terms of reference for this report were modeled after those developed for the H_2S review with changes as required to address SO_2. One exception is the "low-dose" term of reference. As explained previously, exposure concentrations higher than 100 ppm were included in the SO_2 review to capture a complete picture of the potential health effects of SO_2 exposure. In practical terms, only non-clinical animal studies reported measured SO_2 concentrations greater than 100 ppm. Another exception concerns exposure times longer than the defined "short-term" exposure of 30 days or less. Some studies exposed animals for longer time periods. However, if health effects occurred within 30 days, the effects were considered a result of "short-term" exposure and were included in the review.

The terms of reference as outlined in the H_2S report and adapted for the SO_2 report are:

- The review was to focus on the health effects following short-term exposure. The term "*short-term*" was to include exposures of both an acute and subacute variety, to capture exposures lasting a few hours to a few days. The subacute category was further defined to include exposures extending up to 30 days.
- The review was to focus on health effects *per se*. Although a formal definition of "*health effects*" was not adopted by the Expert Panel, the meaning was taken to be: *An undesirable or harmful effect on an organism with adverse consequences affecting survival, growth, development, performance, structure and/or function.*
- The review was to be limited to peer-reviewed, scientific publications. Preference was to be given to English-language journals.
- The review was to include all currently and readily available journal articles, with a strict need to avoid possible journal and/or sponsor bias.
- The review was to focus on scientific studies involving exposures to SO_2 via inhalation to mimic the expected route of exposure of the general public. Studies involving other routes of exposures (e.g. oral, dermal, injection) were to be excluded from review.
- The review was to focus on full-length primary scientific investigations involving controlled exposures of human subjects in laboratory settings, non-clinical studies involving controlled exposures of test animals in the laboratory and "population" studies involving exposures following routine or accidental releases of SO_2 into the environment.
- The review was to include a critical assessment of the technical quality of each scientific paper based on

consideration of experimental design, conduct and reporting. Judgment of quality was to be based on comparison against testing protocols recommended by leading scientific authorities.

A difference between this report and the H_2S report is the inclusion of epidemiology studies in this report. Epidemiology studies investigating a link between short-term changes in exposure and short-term changes in health effects were included in the report. The inclusion of epidemiology studies substantially increased the number of studies included in this report.

Consistent with the H_2S review, *this SO_2 review is strictly a scientific exercise. Only the technical criteria and the scientific meanings of the findings will be presented. Issues of public health implications or policy setting are beyond the scope of this review.*

A large number of studies investigated effects on hypersusceptible subjects (those with asthma, chronic obstructive pulmonary disease or hypersensitive airways). These studies were included in addition to those observing healthy or normal subjects.

The focus of this work was the assessment of the body of scientific knowledge on the potential health effects to humans following short-term exposure to SO_2. To that end, human and animal toxicology studies were included in this review. Effects on livestock were not considered, as they are the subject of a separate review spearheaded by Alberta Environment. The sole exception is two studies on allergic sheep, which were deemed to be of a toxicological nature.

IV. METHODS

As with the H_2S report, work on this report followed several defined stages. The first stage was the literature search. A preliminary search was initiated by Cantox Environmental Inc. The preliminary results formed part of the basis for a more extensive literature search by Alberta Health and Wellness. The purpose of the extensive literature search was to ensure all relevant studies were identified and ultimately included in the final report. Following the search and collection of the literature, a Reference Manager database was created. This served to assign to each study an identifying number for the purposes of the report. The inclusion of key words in the database allowed for the identification of studies focusing on various health effects. The studies were then reviewed following the quality criteria established for the H_2S report. The review team represented a variety of scientific and/or epidemiological backgrounds and each had substantial experience critically reviewing scientific literature. A rating based on the quality criteria (low, moderate or high quality) was assigned to each study by the review team. The next step involved the interpretation of the studies, with the emphasis on studies rated moderate or high.

A. SEARCH STRATEGY

The search strategy was similar to the H_2S search, but more refined with respect to the search terms used, due to the fact that the Term of Reference had been established before the literature search began. A full list of search terms is given in Appendix 2. The eligibility criteria for the SO_2 search were the same as for the H_2S search were established by the members of the Expert Panel and reflect the Terms of Reference. A difference in eligibility criteria for the studies was that in the SO_2 search there was no limit on the exposure concentration. The electronic search used the DIALOG Information Retrieval Service, which includes the following databases:

BIOSIS REVIEWS (1993-2002)
ENVIRONMENTAL BIBLIOGRAPHY (1966 to 2002)
ENVIROLINE (1975 to 2002)
LIFE SCIENCES COLLECTION (1982 to 2002)
MEDLINE (1966 to 2002)
POLLUTION ABSTRACTS (1970 to 2002)
TOXFILE (1966 to 2002)

B. SO_2 DATABASE

To organize and catalogue the large number of studies found in the initial search, all studies were entered into a Reference Manager electronic database. In addition to bibliographic information, keywords and the abstracts of all the papers were incorporated into the database. The keywords corresponded to health effects that were to be highlighted in the final report. The unique identifying numbers assigned to the studies as well as the keywords in the database allowed for the easy location of studies pertaining to specific health effects.

Before entry into the database, studies were subjected to a preliminary assessment as to whether they fulfilled the criteria of acute exposure to SO_2 alone. Studies assessing and reporting the results of SO_2 exposures in combination with other compounds (e.g. PM, smog, etc.) were not included. Each of the 400+ studies in the database were reviewed independently by three members of the review panel. Upon completion of the detailed review, several studies were found not to conform to the eligibility criteria of the study. Of the over 400 studies entered into the database, 347 were included in the final report.

C. QUALITY CRITERIA

All of the over 400 studies reviewed for this report were assessed against the quality criteria established by the expert panel for the H_2S report. The review templates developed for the H_2S report were used in this review (see Appendix 4). During the review, each study was judged and ranked according to its technical quality as determined by the quality criteria. The ranking categories, also called the "confidence index ranking", were identical to the categories used to rank studies in the H_2S report. The following descriptions of the rankings are taken directly from the H_2S report[5]:

High – Signifying that the study meets or exceeds the recommended guidelines, with no serious weaknesses in experimental design, conduct or reporting. Procedures are well-described and results are properly disclosed to permit meaningful interpretation. Study validity is obvious. Confidence in the findings and conclusions is high.

Moderate – Signifying that the study generally subscribes to the recommended guidelines, but minor deficiencies in design, conduct or reporting detract from the interpretation of the results. Study validity is evident, but not obvious. Careful attention to detail in describing procedures and presenting results and conclusions is somewhat restrained, but not weak.

Low – Signifying that the study fails to meet the recommended guidelines and serious weaknesses in design, conduct and reporting are evident. Significant departures from the recommended guidelines may be present. Sufficient detail is lacking to permit meaningful interpretation of results. Study validity is questionable. Confidence in the findings and conclusions is low."

Of the 347 studies included in the final report, 184 (53%) were rated to be of "low" quality, 149 (43%) were ranked "moderate", and 15 (4%) were judged to be of "high" quality.

D. REVIEW PROCESS

A total of seven reviewers made up the review team for this report. Each paper was reviewed independently by three members of the review team with the goal being to eliminate or reduce reviewer bias. The three reviews for each paper were then combined into a single review. The reviewers represented a variety of scientific and epidemiological backgrounds and each had substantial experience critically reviewing scientific literature. The majority of the reviewers had graduate degrees (Master's level or higher). The papers were assigned to the reviewers in a staggered fashion by study ID such that groups of papers were reviewed by different sets of three reviewers.

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[5] Health Effects Associated with Short-term Exposure to Low Levels of Hydrogen Sulphide (H_2S) – A Technical Review; Cantox Environmental Inc., 2002

OTHER IMPORTANT NOTES:

- SO_2 is frequently used to induce bronchoconstriction in human and animal studies testing asthma medications. Studies of this type were included if the effect of SO_2 alone could be determined in the study separately from the effect of the medication being tested.

- No exposure concentration limit was set on the studies included in this review, with the purpose being to provide a complete overview of the peer-reviewed scientific literature and a full picture of the health effects of SO_2 exposure.

- Some non-clinical studies report exposure longer than 30 days (the cut-off point for "short-term" as defined by the Expert Panel). These studies are included in this review if effects were seen at time periods shorter than the full exposure reported in the study.

- In the H_2S report, human population studies were termed "case-control". In this report, human population studies and case reports are termed epidemiology studies.

- Some studies reported concentrations in units other than ppm or ppb. All units have been converted to ppm or ppb for consistency and to facilitate comparison. The equation used for the conversion was:

  $\text{ppm} = \text{mg/m}^3 \text{x } 24.45/\text{mol. wt.}$

Where:
24.45 is the volume of 1 mole of air at 25°C and 1 atmosphere; and
mol. wt. is the molecular weight of SO_2 = 64.06

V. SUMMARY OF HEALTH EFFECTS

A. Abbreviations

APHEA = Air Pollution and Health, A European Approach
BAL = bronchoalveolar lavage
COPD = chronic obstructive pulmonary disease
C/P ratio = cholesterol/phospholipid ratio
FEV$_1$ = forced expiratory volume in 1 second
FVC = forced vital capacity
FEF$_{25}$ = maximum flow rate at the last 25% of the vital capacity
FEF$_{50}$ = maximum flow rate at the last 50% of the vital capacity
FRC = functional residual capacity
IP = intratracheal pressure
MEF$_{50\%VC}$ = maximum expiratory flow from one half vital capacity
MMF = maximum mid-expiratory flow
MMFR = maximum mid-expiratory flow rate
NO$_2$ = nitrogen dioxide
PEF = peak expiratory flow
PM$_{10}$ = particulate matter with an aerodynamic diameter of less than 10 μm
Rl = pulmonary flow resistance
Rn = nasal flow resistance
R$_T$ = total respiratory resistance
SO$_2$ = sulphur dioxide
^{35}SO$_2$ = radiolabelled SO_2
SRaw = specific airway resistance
V$_{max50}$ = maximum flow calculated at 50% vital capacity
V$_{max75}$ = maximum flow calculated at 75% vital capacity

B. Overview

The goal of this review as laid out in the Term of Reference was to evaluate the scientific literature on health effects of short-term exposure to SO_2. "Short-term" was defined as being from a few minutes to 30 days. Some studies reported exposures lasting longer than 30 days, but with effects apparent in 30 days or less. No upper limit on concentration was set. Only inhalation exposure was considered.

The technical quality of each study was evaluated against pre-determined quality criteria. These quality criteria were developed for the H_2S review and are equally applicable to this review. Since fully transferable criteria from the expert panel were available by which to judge the quality of the SO_2 literature, it was not necessary to develop new criteria for this report.

Each study was reviewed independently by three members of a seven-member review panel. Each member of the review panel conducted their review cogniscent of the need for objectivity, consistency, and fairness.

Each study was ranked according to the level of confidence in the results, also called a "Confidence Index Ranking". This ranking was achieved by a careful evaluation of the strengths and weaknesses of the study. Written summaries of the studies followed a pre-determined format. These written summaries can be found in Appendix 6.

A summary of the health effects of acute exposure to SO_2 is presented in the balance of this chapter. The results are organized on a system-by-system basis similar to the organization of the H_2S review. In the interpretation of the review results, emphasis was placed on studies ranked "high" or "moderate". Studies ranked "low" were considered to have too many weaknesses in study design or reporting to provide reliable evidence of health effects and were therefore not emphasized in the interpretation.

Confidence Index Ranking
High or high-to-moderate ▲
Moderate-to-high, moderate or moderate-to-low ◆
Low or low-to-moderate ●

The 347 studies included in the review were assessed based on their technical quality. Only 15 studies (4%) were judged to be of "high" quality with no major flaws in study design or reporting. 149 studies (43%) were found to be of "moderate" quality with some weaknesses in either study design or reporting. 183 studies (53%) were judged to have major weaknesses in study design or reporting and were classified as being of poor or "low" quality. Common weaknesses in those studies rated "moderate" or "low" were very similar to the weaknesses reported in the H_2S literature.

Summary tables are provided at the back of this section. These tables list the positive (health outcomes were reported) and negative (health outcomes were not reported) findings presented in the human clinical, non-clinical animal and human epidemiology studies according to the concentrations at which these results were observed. The level of confidence in the findings of each study is indicated. Figures detailing concentration and effect associations at various time intervals are also presented. These tables and figures provide a visual interpretation of the weight-of-evidence provided by the reviewed human clinical and non-clinical animal literature. *These tables and figures do not stand alone and should not be interpreted in the absence of the corresponding written summary.*

No effort has been made to extrapolate results from the animal studies to humans. Such an extrapolation would require knowledge and assumptions beyond the scope of this review of the scientific literature.

C. General Comments

Studies were grouped by study type: human clinical, animal non-clinical, and human epidemiology. Clinical studies were those in which human subjects were exposed to SO_2 under strictly controlled conditions. Non-clinical studies were similar, with the use of animals rather than human subjects. Epidemiology studies included population studies in which the uncontrolled exposure and possibly corresponding health effects of large populations was observed, and case-reports in which very few subjects experienced accidental exposure to high, unmeasured levels of SO_2.

SO_2 is frequently used to induce bronchoconstriction in human and animal studies testing asthma medications. This was the main focus of some of the studies reviewed. These studies were included because the effect of SO_2 alone could be determined in the study separately from the effect of the medication being tested.

Several common weaknesses were observed in the studies reviewed. These weaknesses were very similar to those found in the H_2S literature.

Large numbers of studies used only one concentration. One of the criteria for a causal association between an exposure and an effect is the presence of a dose-response relationship. It is impossible to determine a dose-response relationship from only one exposure concentration. Therefore, these studies had limited contribution to the assessment of SO_2-related health effects.

Many clinical and non-clinical studies tested subjects of only one gender, predominantly males. The use of only one gender limits the generalizability of the results to larger populations.

In many non-clinical studies there was a lack of conventional study designs, particularly Good Laboratory Practices and standard measures of toxicity. None of the studies specifically reported following Good Laboratory Practices. When standard practices are followed, the reliability of and confidence in the results of a study are greatly increased. In addition, interpretation of the clinical significance of observations is facilitated. Many studies failed to report standard measures of toxicity such as signs and symptoms, pathology, and body weights.
The goal of many studies was to investigate the mechanism of action of SO_2 in inducing health effects, particularly with respect to the biochemistry of the respiratory system. These studies reported many results at the subclinical level. The clinical significance of these results is unclear and often not discussed in the individual studies.

Both the epidemiology studies and the case reports showed a general lack of good exposure assessment. Case reports involve traumatic, accidental exposures to high concentrations of SO_2 and as such, accurate exposure concentrations are seldom measured and reported. This makes a quantitative evaluation of the exposure-effect association impossible. However, more qualitative interpretations of these reports can be useful. For example, these reports record severe human health effects observed after exposure to concentrations much higher than would be ethically possible in an experimental situation. The case reports of accidental exposure to "very high" concentrations of SO_2 therefore give us qualitative information on the effects of extreme exposures.
The epidemiology studies are subject to a limitation common to many environmental epidemiology studies: that of inaccurate exposure assessment. Most of the studies reviewed looked at exposure-effect relationships on the most general population level with both exposure and effects identified only for populations, not individuals. In these studies it is impossible to determine whether those individuals presenting with health effects are the same individuals that were exposed. This is called the "ecological fallacy". Other studies evaluated individual health effects, but relied on a few ambient monitors for exposure measurements. Concentrations of air-borne contaminants vary depending on wind speed and direction at any given time, as well as the presence or absence of point sources. Making a link between an individual health effect and this tenuous

measure of individual exposure is difficult. The results of these studies consequently must be evaluated with care.

Please note that the following descriptions of health effects are summaries only. For more detailed information on the design and reporting of the studies reviewed in this report, readers are encouraged to examine the written reviews of individual studies found in Appendix 6 (an electronic attachment).

D. Mortality

Few studies investigated mortality at low levels of exposure. All studies investigating or reporting mortality at all levels of short-term exposure are included here.

Clinical studies

Understandably, no clinical studies investigated mortality outcomes due to ethical considerations.

Non-clinical studies

Azoulay-Dupuis et al. (1982) observed significant increases in mortality rate and significant decreases in survival time in mice with a bacterial infection exposed to 10 ppm SO_2 for durations of one week or longer (Study ID ▲ 172).
Grose et al. (1986) observed no significant change in mortality caused by bacterial infection in mice exposed to 0.95 ppm SO_2 for 2 hours and subsequently challenged with Streptococcus (Study ID ◆ 174).
Bitron and Ahronson (1978) observed increases in percent death in mice during exposure and cumulated mortality with increasing exposure time at concentrations of 900, 1400, and 1900 ppm for exposure times between 10 and 640 minutes (Study ID ◆ 224).
Hilado and Machado (1977) calculated the LC_{50} values for various concentrations and exposure times for Swiss albino mice as: 6800 ppm at 5 minutes, 4400 ppm at 10 minutes, 4000 ppm at 15 minutes and 3000ppm at 30 minutes. However, the number of mice in this study was not clearly reported and the study did not follow Good Laboratory Practice guidelines. (Study ID ● 284).

Asmundsson et al. (1973) observed no increased mortality in hamsters or rats exposed to gradually increasing concentrations up to 400 ppm SO_2 for 5 hours per day, 5 days per week, for 6 weeks. However, in the rats exposed abruptly to 400 ppm, three of 30 rats died in the first 5-hour exposure and 22 of the remaining rats died within the first week of exposure. All six of the hamsters exposed to 400 ppm died in just over 6 hours of exposure (Study ID ● 198).
Cohen et al. (1973) observed decreasing survival time of rats exposed to SO_2 concentrations of 590 ppm and higher with increasing exposure time up to a maximum exposure time of 4 hours (Study ID ● 218).

Fedde and Kuhlmann (1978) exposed chickens to concentrations from 1 to 5000 ppm SO_2 for 60 minutes. Death occurred rapidly in almost all birds at 5000 ppm. However, only two birds in ten died when exposed to 1000 ppm. No deaths were observed at lower concentrations (Study ID ▲ 183).

Epidemiology studies

Many epidemiology studies and case reports have investigated the association between SO_2 exposure and mortality. Theses studies are summarized in Tables 4A to 4D. The majority of these studies are ecological in nature with outcomes determined on an individual level and exposure determined at a population level. Subsequently, the major weakness observed in these epidemiology studies is the potential for exposure misclassification as a result of the exposure assessment methods. Because of this substantial limitation, the confidence in the results and conclusions from these epidemiology studies could not be judged to be higher than moderate and in most cases the confidence was judged to be low. Many of these studies employed a time-series analysis, meaning that the study was conducted over a period of time. However the focus of these investigations is on short-term changes in SO_2 concentrations (generally, daily) resulting in short-term changes in health status.

All-cause or total mortality

Katsouyanni et al. (1997) pooled the results of the APHEA (Air Pollution and Health, A European Approach) study looking at association between all-cause mortality and concentrations of SO_2 (and other air pollutants) in 12 European cities. Pooled results suggest that a 19 ppb increase in SO_2 is associated with an increase of 3% (95% CI 2% - 4%) in daily mortality in Western European cities and a 0.8% (95%CI -0.1% - 2.4%) increase for central eastern European cities (mean winter SO_2 concentrations 11.4-125.9 ppb). Significant heterogeneity was observed in the SO_2 results (Study ID ◆ 336).

Xu et al. (1994) estimated that the risk of all cause mortality increased by 11% (95% CI 5% -16%) with a doubling of SO_2 concentrations (mean 39 ppb; maximum 240 ppb) in Beijing, China in 1989. A doubling of SO_2 was also associated with significant increases in death from chronic obstructive pulmonary diseases, pulmonary heart disease, and cardiovascular disease (Study ID ◆ 338).

Sunyer et al. (1996) reported that a 38 ppb increase in SO_2 concentrations (in Barcelona, Spain during the period 1985 to 1991 was statistically significantly associated with total mortality (RR 1.13), elder mortality (≥70 years; RR 1.13), and cardiovascular mortality (RR 1.14) for the whole year and the winter, and total mortality, elder mortality, cardiovascular mortality, and respiratory mortality for the summer. SO_2 concentrations ranged in winter from 0.8 ppb-61.1 ppb (mean 17.6 ppb) and in summer from 2.1 ppb-44.5 ppb (mean 13.9ppb). (Study ID ◆ 345).

Touloumi et al. (1996) investigated the effects of short-term effects of "winter type" air pollution (range 2.3 – 138 ppb) on the daily total number of deaths in Athens during 1987 to 1991 as part of the APHEA project. They observed a statistically significant association between SO_2 concentration increases of 38 ppb and a 12% (RR 1.12 (5%CI 1.07-1.16) increased risk of daily mortality (Study ID ◆ 349).

Dab et al. (1996) observed significant associations (RR 1.085 95%CI 1.015-1.159) between mean daily 24-hour SO_2 concentration increases of approximately 38 ppb (above the 5th centile of 2.6 ppb) and daily count of deaths in Paris as part of the APHEA project (Study ID ◆ 351).

Zmirou et al. (1996) investigated the association between daily mortality and ambient air pollution in Lyon, France as part of the APHEA project. Significant associations were observed with a 19 ppb increase in SO_2 (mean 46.76 ppb; range 2.12-315 ppb) for total mortality (RR 1.06 95%CI 1.02-1.09) minus external causes, respiratory deaths, and cardiovascular deaths for the time period between 1985 and 1990 (Study ID ◆ 352).

Using autoregression models, Touloumi et al. (1994) reported a significant association ($p<0.001$) between total daily mortality and the log of air pollution concentrations in Athens, Greece between 1984 and 1988. They state that a 10% reduction in SO_2 would be estimated to decrease mortality by 0.65%. Complete daily data on cause of death and age were not available over the whole period of the study. 24-hour mean SO_2 concentrations were 17±12 ppb (Study ID ◆ 361).

Wietlisbach et al. (1996) investigated the association between mortality and air pollutants in Zurich, Basle, and Geneva, Switzerland between 1984 and 1989. They reported several significant associations with a 3-day moving average of SO_2: total mortality, cardiovascular mortality, and elder mortality (≥65 years) in Basle and Geneva; respiratory mortality in Zurich and Geneva. However, in Basle, the association between SO_2 and mortality was negative at the highest levels of SO_2. Associations and confidence levels were not reported. SO_2 concentrations were: Zurich: 35.4±35.5 ppb, Basle: 26.5±25.3 ppb, Geneva: 40.2±32.7 ppb (Study ID ◆ 403).

Wong et al. (2001) assessed the effects of air pollution on mortality in Hong Kong. Significant associations between SO_2 concentrations and mortality were observed in the cool season (Total mortality RR: 1.04 95% CI: 1.02 – 1.07; Respiratory mortality RR:1.04 95% CI: 1.00 – 1.09; CV mortality RR:1.07 95% CI: 1.02 – 1.11), but not the warm season (Total mortality RR: 1.02 95% CI: 0.99 – 1.04; Respiratory mortality RR: 1.02 95% CI: 0.99 – 1.09; CV mortality RR: 1.01 95% CI: 0.97 – 1.05). This is of interest, because the average SO_2 concentrations were very similar between the two seasons: 6.5 ppb (cool) and 6.9 ppb (warm). This result is explained in the study as the result of greater variability of weather in the warm period and activity differences between the two seasons (Study ID ◆ 464).

Buechley et al. (1973) analyzed deaths in New York and Philadelphia compared to daily SO_2 measurements. They observed an increase in mean mortality residuals with increasing SO_2 concentrations. Mortality excesses of 2% were observed on days when SO_2 concentrations were greater than 190 ppb and mortality was 1.5% less than expected on days when SO_2 levels were below 11 ppb (Study ID ● 012).

Moolgavkar et al. (1995) examined daily mortality and air pollution in Philadelphia between 1973 and 1988. The results indicate that a 100 ppb increase in SO_2 (above daily means of 16.8 ppb (spring) and 25.4 ppb (winter)) is significantly associated with an increase in daily mortality in the spring (RR: 1.19 95% CI: 1.06 – 1.33) and winter (RR: 1.21 95% CI: 1.09 – 1.35), but not in fall (daily mean 17.8 ppb; association not reported) and summer (daily mean 15.7 ppb) (Study ID ● 334).

Spix et al. (1993) investigated daily mortality and air pollution in Erfurt, East Germany between 1980 and 1989. They concluded that effects of SO_2 on mortality are small: 10% excess mortality when comparing the 95% quantile (355 ppb) to the 5% quantile (9 ppb) (RR 1.10; $p<0.01$). The authors also conclude that SO_2 concentrations likely do not represent personal exposure (mean 75 ppb; range of means 3.8-1361ppb) (Study ID ● 337).

In a paper discussing different predictive epidemiological model building, Spix and Wiehmann (1996) predicted that daily SO_2 mean concentrations between 8 and 47 ppb would result in an increase in daily mortality of 3% for lag day 1 in Köln, Germany (Study ID ● 348).

Glasser and Greenburg (1971) suggest that increases in mortality are associated with increases in ambient SO_2, independent of weather factors in New York City between 1960 and 1964. Differences in mean numbers of deaths were compared between days with SO_2 concentrations of 200 ppb or less and days with 400 ppb or more. Data analysis and exposure assessment are the main limitations of this study (Study ID ● 357).

Krzyzanowski and Wojtyniak (1991) investigated the association between air pollution and daily mortality in Cracow, Poland in the winter months during the period 1977 to 1989. The authors report an association between SO_2 with concentrations greater than 76 ppb, as well as incremental concentration increases of 38 ppb (above 76 ppb; RR=1.19) and daily mortality. No associations were reported at these concentrations in males older than 65 years. However, there are inconsistencies in the reporting, the data analysis is questionable, and confidence intervals are not given, therefore significance cannot be established (Study ID ● 359).

Schimmel and Greenburg (1972) attempted to predict the number of excess deaths attributable to air pollution in New York City during the years 1963 and 1968. They estimate that approximately 20% of the excess deaths could be attributed to SO_2 at concentrations of 17±11 ppb. However, limitations in the analysis and reporting of the data raise questions as to the validity of the results (Study ID ● 366).

Rahlenbeck and Kahl (1996) reported a 4.5% excess mortality in East Berlin in the winters of 1981 to 1989 for increases of 38 ppb SO_2 with means of 41 to 84 ppb. However, limitations of the study design make it difficult to ascertain whether these associations are in fact real (Study ID● 391).

Burnett et al. (1998) investigated the effect of ambient SO_2 on daily mortality from non-accidental causes in 11 Canadian cities. There was little consistency in the relative risks among the cities. The average increased risk of mortality over all the cities from changes in mean SO_2 concentrations (values not given; mean daily concentration 5.4 ppb) was 1.4 %. Confidence intervals and significance were not reported. No information was reported on monitoring systems in each city or the sensitivity of the monitoring instruments, which is important given the low SO_2 concentrations (daily average: 0.7 to 10.5 ppb). The main focus of the study was on mixtures of pollutants, not single pollutants (Study ID ● 395).

Le Tertre et al. (2002) reported significant associations between a 19 ppb increase in SO_2 (baseline exposure not reported) and total mortality (RR: 1.036 95%CI 1.021-1.052), cardiovascular mortality, and respiratory mortality in a study of 9 French cities between 1990 and 1995. There was substantial heterogeneity in the results and the SO_2 concentration measurements among the cities (Study ID ● 407).

Ha et al. (2003) found some positive associations between a 7.8 ppb increase in SO_2 (Mean: 11.1±7.0 ppb Range: 2.4-46.0ppb) and mortality for some age groups. However, these results are presented but not discussed in the study. The focus of this study is PM_{10} (Study ID ● 408).

Botter et al. (2002) observed a significant 2.4 % increase in the daily death count for people over 65 years old in Sao Paulo Brazil between 1991 and 1993 with a 4 ppb increase in SO_2 for a 3-day lag (baseline exposure 1.9-23 ppb). The authors speculate that this effect may reduce the life span of already frail individuals by a few days (Study ID ● 414).

Schwartz et al. (2001) investigated a dose-response relationship between SO_2 and daily mortality in eight Spanish cities. There was a weak association between an increase of 4 ppb SO_2 (baseline exposure 4.2-17 ppb) and daily deaths (0.27%, 95%CI: 0.18-0.73%). However, the risk is not linear with increasing dose and eventually levels off at 20-30 ppb and declines with further increases in concentration (Study ID ● 419).

Small but significant increases in percentage total deaths (RR: 1.0027 95%CI 1.0018-1.0073) were reported with a standard deviation increase in SO_2 concentrations (range 0.3 – 15 ppb) in the summer in Vancouver, British Columbia by Vedal et al. (2003). Similar observations were made in the winter for lag 1. The increases in total deaths were small and confidence intervals were wide. It is speculated that stratification by season may have resulted in a lack of statistical power or that the extensive smoothing of the data affected the associations (Study ID ● 434).

Alberdi Odriozola et al. (1998) found significant associations between a 38 ppb increase in SO_2 concentration (means: 27±20 ppb and 30±17 ppb) and daily mortality. However, the lag times and significance depends on how the data are analyzed with respect to gender, age, season, and cause of death (Study ● ID 465).

Respiratory mortality

Vigotti et al. (1996) observed an increased risk of respiratory death (RR = 1.12, 95% CI 1.03, 1.23) with increased daily SO_2 concentrations of 1 to 316 ppb in Milan, Italy (Study ID ◆ 027).

Zeghoun et al. (2001) observed associations between interquartile increases in SO_2 and respiratory mortality in Rouen, France (IQR increase = 7-14 ppb; Rouen: 8.2% increase 95% CI: 0.4%– 16.6%) and cardiovascular mortality in Le Havre, Franc (IQR increase = 4-13 ppb; 3% increase 95% CI: 0.8% – 5%). Baseline concentrations of SO_2 were: Rouen: Summer: 9.1 ppb, Winter: 13.5 ppb; Le Havre: Summer: 10.6 ppb Winter: 15.1 ppb. Small numbers of deaths were observed and confidence intervals were large (Study ID ◆ 430).

Derriennic et al. (1989) conducted a study in two French cities investigating the possible link between SO_2 air pollution and mortality. They observed a statistically significant association between daily SO_2 concentration and respiratory deaths up to 10 days later in the age group 65 years and older. No relationship was seen between SO_2 concentration at averages of 19 or 25 ppb and cardiovascular deaths (Study ID ● 002).

Hong et al. (1999b) found that SO_2 concentrations above 40 ppb, but not below 40 ppb were a significant predictor of respiratory mortality with lag day 1, but not with total or cardiovascular mortality in Inchon, Korea over a 20-month period from January 1995 through August 1996 (Study ID ● 412).

Wong et al (2002) observed barely significant associations between a 4 ppb increase in SO_2 (mean 6.4±4.4 ppb) and respiratory mortalities or ischaemic heart disease (RR: 1.015 95% CI: 1.001 – 1.029). The magnitude of the effects was very small (Study ID ● 422).

Venners et al. (2003) observed statistically significant associations between respiratory and cardiovascular mortality and a 38 ppb increase in mean SO_2 concentrations (mean 81.2 ppb) in Chongqing, China in 1995. The associations were strongest on the second and third lag days (Respiratory RR (2d lag): 1.11, 95% CI: 1.02 0 1.22; CV RR (2d lag): 1.10, 95% CI: 1.02 – 1.20; (3d lag): 1.20, 95% CI: 1.11 – 1.30) (Study ID ● 461).

Stroke mortality

Hong et al. (2002b) found significant increased risk (RR: 1.04, 95% CI: 1.01 – 1.08) of ischemic stroke mortality for each interquartile range increase in SO_2 (17.43 ppb; mean 22±19ppb) in Seoul, Korea over a 7-year period (January 1991 to December 1997). However, the results for hemorrhagic stroke mortality were not significant (Study ID ● 397).

Hong et al. (2002a) report a 2.9 % (95%CI: 0.8-5.0%) increase in stroke mortality with a 5.7 ppb increase in SO_2 (Mean: 12.1 ± 7.4) for a 2-day lag in Seoul, Korea. The authors state that it is difficult to determine in this study whether the increase in stroke mortality truly represents an increase of stroke mortality or only an earlier death by a few days or weeks, of those already about to die from previous strokes or other causes (Study ID ● 415).

No association observed

Verhoeff et al. (1996) investigated the association between air pollution and daily mortality in Amsterdam between 1986 and 1992. No association was found between a 38 ppb increase in SO_2 (mean 5 ppb; max. 53 ppb) and daily mortality regardless of lag day (Study ID ◆ 377).

Simpson et al. (1997) observed no significant association between daily mortality and SO_2 concentrations in Brisbane, Australia using the APHEA protocol. SO_2 concentrations were very low (maximum hourly: 60 ppb) (Study ID ◆ 458).

Mazumdar et al. (1982) examine the relationship between daily deaths and daily concentrations of smoke and SO_2 in London, England for the winters of 1958 to 1972. No significant associations were observed per mg/m^3 (380 ppb) increase in SO_2 (range of means: 69-160 ppb) (Study ID ● 332).

Wojtyniak and Piekarski (1996) reported inconsistent associations between SO_2 concentrations (11 to 28 ppb) and cardiovascular mortalities in four Polish cities. Associations were either not significant or significant, but in both directions (positive and negative). These inconsistent results may be a result of exposure and outcome data discrepancies between cities. This is one of the weakest of the APHEA studies (Study ID ● 350).

Bacharova et al. (1996) observed no significant association (Total mortality RR: 0.998; 95% CI: 0.96 – 0.99) between SO_2 concentrations (Winter:16.3±18.1ppb; Spring:7.7±7.2 ppb; Summer:4.5±1.9 ppb; Fall:7.8±6.4 ppb) and daily number of deaths for any season in Bratislava, Slovak Republic between the years 1987 and 1991. This study followed the APHEA protocol; however, sample size and lack of detail in the reporting limit confidence in these results (Study ID ●354).

Ballester et al. (1996) assessed the short-term relationship between daily air pollution and mortality in Valencia, Spain over the period 1991-1993. No significant results were reported between 4 ppb increases in SO_2 (mean: 15.2±5.9 ppb) and daily mortality (Total mortality RR: 1.007, 95% CI: 0.999 – 1.015; Total mortality (>70yrs) RR: 1.009, 95% CI: 1.00 – 1.21; CV mortality RR: 1.012, 95% CI: 0.995 – 1.026) (Study ID ● 355).

Ballester et al. (2002) investigated the impact of air pollution on mortality in 13 Spanish cities (EMECAM study). For single city analysis, no statistically significant associations were reported between SO_2 concentrations and daily mortality. When the cities were combined, the authors report that a 4 ppb increase in SO_2 (daily mean range: 3.1-17 ppb) is associated with a 0.5% increase in daily deaths; however, there are some inconsistencies in the reporting (Study ID ● 400).

Mackenbach (1993) reported that the positive regression coefficient for the effect of SO_2 on mortality dwindles to zero when all potential confounding factors are taken into account. However, levels of SO_2 measured were reported to be relatively low (range: 5-9 ppb), and the study did not account for the lagged effects of temperature (Study ID ● 356).

Anderson et al. (1996) report a significant association between increased ambient SO_2 concentrations (7-17 ppb) and all-cause mortality in the warm season in London between 1987 and 1992. However, the 95% CI for this association (RR=1.01) includes 1.00 (95%CI 1.00-1.03). No other associations were observed for SO_2 and daily mortality (Study ID ●365).

Kelsall et al. (1997) reported non-significant associations between 12.9 ppb increases in SO_2 concentrations (mean 6.6±4.4 ppb) and total mortality (RR=1.08, 95%CI 0.37-1.78). The focus of this study was TSP rather than SO_2; however, some SO_2 results were reported (Study ID ●389).

In an ecological study, Bobak and Leon (1992) reported a statistically significant association between the highest to lowest quintile for SO_2 (range: <5 to >22 ppb) and post neonatal respiratory mortality. However, confidence intervals included 1 (RR: 3.91 95% CI: 0.90 – 16.9) p=0.062). Only weak, non-significant associations were reported for other infant mortalities (Study ID ● 440).

Saldiva et al. (1994) observed no association between SO_2 concentrations (mean: 6 ±4 ppb) and respiratory mortality in children in Sao Paulo, Brazil for the period May 1990 to April 1991 (Study ID ● 442).

Kinney and Ozkaynak (1991) observed no association between changes in SO_2 concentration (mean 15 ± 6 ppb) and mortality in Los Angeles County, California for the time period 1970 to 1979 (Study ID ● 443).

Kotesovec et al. (2000) found no association between daily total mortality and 38 ppb increase in SO_2 concentrations (mean 38±34 ppb) for the entire population of Northern Bohemia when gender, age, and cause of death were not separated out. In the over 65 years age group, higher daily cancer mortality rates in males were associated with increased SO_2 concentrations (Study ID ● 479).

Hong et al. (1999a) investigated the association between total daily mortality or cardiovascular mortality and air pollution, including TSP, PM_{10}, SO_2, NO_2, O_3, and CO in Inchon, Korea. Relative risks for an increase of 4 ppb in SO_2 (mean 22.6 ppb) were not significant for either total or cardiovascular mortality (Total mortality RR: 1.007, 95% CI: 0.56 – 1.062; CV mortality RR: 1.028, 95% CI: 0.937 – 1.129) (Study ID ●480). Schwartz and Dockery (1992) investigated the association between total mortality and total suspended particulates (TSP) and SO_2 in Philadelphia. Deaths from accidents and deaths outside the city were excluded and possible confounders such as year, season, temperature, and humidity were controlled for. A significant positive association was observed between total mortality and SO_2 for both current day and prior day SO_2 measurements. Total mortality was estimated to increase by 5% with each 38 ppb increase in SO_2 (mean: 21 ppb). However, when TSP and SO_2 were considered simultaneously, the SO_2 association was no longer significant (Study ID ● 483).

Case-studies

A few studies investigated industrial accidents where humans were exposed to extremely high levels of SO_2 in catastrophic circumstances. Exposure concentrations are generally not measured in these situations. Harkonen et al. (1983) report the experiences of seven men accidentally exposed to high concentrations of SO_2 in a pyrite mine explosion. Exposure concentrations are unknown and duration of exposure was estimated at 20 to 25 minutes. Nine men were initially exposed. Two subsequently died. (Study ID ● 021). A case report (Charan et al., 1979) describes an industrial accident in which five men were exposed to very high, but unmeasured, concentrations of SO_2. Two of the men died and the short-term and long-term symptoms of the remaining three men are described in detail (Study ID ● 270).

Summary:

Clinical

No clinical studies used mortality as a health endpoint, for ethical reasons.

Non-Clinical

There are few high or moderate ranked non-clinical studies on mortality. Of those, one high quality study found an increase in mortality rate and decreased survival time of mice with bacterial infections after exposure to 10 ppm SO_2 for one week or longer. However, a moderate quality study reported no change in mortality from bacterial infection in mice exposed to 0.95 ppm for two hours. Two studies observed increases in mortality rates in mice or chickens with increasing exposure time and SO_2 concentration. In mice the SO_2 concentrations ranged from 900 to 1900 ppm for times of 10 to 640 minutes. For chickens, the concentrations ranged from 1 to 5000 ppm for 60 minutes with deaths occurring above 1000 ppm. One low quality study attempted to determine the LC_{50} in mice at various concentrations and time. However, this study had many limitations, including failure to follow Good Laboratory Practice guidelines.

Epidemiology

Many epidemiology studies and case reports investigated an association between SO_2 exposure and mortality.

The majority of these studies are ecological in nature with outcomes determined on an individual level and exposure determined at a population level. The exposure data collected is generally of ambient levels. Since humans spend a large portion of their time indoors and travel through various micro-climates during their daily activities, ambient levels will likely not be a good measure of exposure at the individual level. Subsequently, the major weakness observed in these epidemiology studies is the potential for exposure misclassification as a result of the exposure assessment methods. Much of the exposure and outcome data used in these studies is retrospective and from public records, which increases the probability of misclassification and bias. Many confounding factors cannot be accounted for when using these types of data.

In addition, SO_2 is just one element in a mixture of pollutants found in "air pollution". It is difficult to isolate the effects of SO_2 from those of other single pollutants or combinations of pollutants. Because of these substantial limitations, the confidence in the results and conclusions from these epidemiology studies could not be judged to be higher than moderate and in most cases the confidence was judged to be low.

Keeping these limitations in mind, general conclusions can be extracted from the moderate quality studies. Several European studies observed an association between in increase of 38 ppb SO_2 and daily all-cause mortality. These studies were part of the APHEA (Air Pollution and Health, a European Approach) project. One of the APHEA studies, however, observed no association between a 38 ppb increase and mortality. There was substantial variation in results among the APHEA participant cities.

Other studies suggest an association between SO_2 concentrations ranging from 19 and 41 ppb and daily all-cause mortality. An Australian study observed no association between daily mortality and SO_2 in Brisbane with maximum hourly concentrations of 60 ppb.

Other studies observed small but significant increases in respiratory mortality in Milan (1-316 ppb) and Rouen, France (increase from 7 to 14 ppb).

E. Respiratory System

Please refer to Tables 1 to 9 and Figures 1 to 7 for further summary of the information in the following section.

Respiratory System - Functional

Clinical studies

Numerous clinical studies investigated the effects of acute SO_2 exposure on respiratory function. These studies have been arranged in subcategories for clarity and comparison.

Adolescents

One group, Koenig et al., carried out a suite of studies investigating the respiratory effects of SO_2 on adolescents. In one study, (Koenig et al., 1982a) eight adolescents with signs of hyperactive airways but no clinical diagnosis of asthma were exposed to 1 ppm SO_2 for 30 minutes at rest and 10 minutes while exercising. Baseline function values did not change significantly. However, statistically significant reductions (24 to 34%) were observed in FEV_1, V_{max50}, and V_{max75} after exercise (Study ID ◆ 038).

In a related study (Koenig et al., 1982b), healthy adolescents were exposed to SO_2 using the same exposure protocol. No statistically significant changes to pulmonary function parameters were observed after exposure at rest. Slight, but statistically significant reductions were observed in FEV_1, V_{max50}, and V_{max75} after exercise (Study ID ◆ 042). In a further study (Koenig et al., 1985), asthmatic adolescents were exposed to 0.5 ppm SO_2 for 50-minute periods on five separate days to investigate the relationship between changes in nasal power and pulmonary function depending on the route of exposure to SO_2 (oral or oronasal). Statistically significant changes (16-69%) in total respiratory resistance, FEV_1, V_{max50}, and V_{max75} were observed for all routes of exposure. In addition, changes in FEV_1 and V_{max50} were greater after oral inhalation compared to oronasal inhalation (Study ID ◆ 099).

The research group (Koenig et al., 1987) exposed asthmatic adolescents to 0.75 ppm for 10 minutes during exercise and observed a statistically significant decrease in FEV_1 and an increase in total respiratory resistance (Study ID ◆ 103).

In a similar study (Koenig et al., 1989), asthmatic adolescents were exposed to 1.0 ppm SO_2 for 10 minutes during exercise (Study ID ◆ 102). Results were similar to those observed in Koenig et al. (1987) (Study ID ◆ 103).

Finally, adolescent asthmatics were exposed to 100 ppb SO_2 for 15 minutes and a slight decrease in FEV_1 and V_{max50} was observed (Koenig et al., 1990; Study ID ◆ 277).

Effects observed-healthy subjects

A multitude of studies reported SO_2-induced effects on pulmonary function in healthy subjects.

Stacy et al. (1981) exposed healthy male subjects to 0.75 ppm SO_2 for 2 hours with one 15-minute exercise period while measuring a variety of pulmonary function parameters. Only airway resistance was significantly changed by exposure to SO_2. Subjects who responded to allergen skin tests were also found to be significantly more reactive to SO_2 than subjects who did not respond to allergen skin tests (Study ID ▲ 060).

Kulle et al. (1986) observed small but statistically significant decreases in spirometric function after exposure to 1 ppm SO_2 for 4 hours per day, three days per week for 3 weeks in healthy adults (Study ID ▲ 096).

Newhouse et al. (1978) exposed healthy adults to 5 ppm SO_2 for 2.5 hours and assessed pulmonary function. They observed a significant decrease in maximum mid-expiratory flow rates and a significant increase in bronchial clearance. No changes in FEV_1 were observed (Study ID ◆ 045).

Bedi et al. (1984) investigated the threshold concentration of SO_2 for pulmonary function changes by exposing healthy adult males to 1 to 2 ppm for 2 hours during which time there were three 30-minute exercise periods. The only significant change observed was an increase in specific airway resistance (SRaw) at both concentrations. No changes were observed in other pulmonary function parameters (Study ID ◆ 047).

Wolff et al. (1975) exposed healthy adults to 5 ppm SO_2 for 3 hours and measured pulmonary function parameters. They observed a significant decrease in maximal midexpiratory flow (Study ID ◆ 056).

Snell and Luchsinger (1969) exposed healthy adults to 0.5, 1, and 5 ppm SO_2

for 15 minutes after a 15-minute control period. They observed a significant decrease in maximum expiratory flow from one half vital capacity ($MEF_{50\%VC}$) at 1 and 5 ppm exposure levels. More exposures at 5 ppm for 15 minutes were done either through a mask or a mouthpiece. Average $MEF_{50\%VC}$ was higher with mask rather than mouthpiece exposure. A reduction in conductance was observed with both exposure methods (Study ID ◆ 070).

Kagawa (1983) exposed healthy adult males to 0.15 ppm of SO_2 for 2 hours. A significant decrease in specific airway conductance was observed in just over half the subjects during exposure. Significant increases were observed in functional residual capacity and residual volume (Study ID ◆ 072).

Andersen et al. (1974) exposed a small group of healthy adults to SO_2 concentrations of 1, 5, and 25 ppm for 6 hours a day for 3 consecutive days. A significant decrease in nasal mucus flow rate was observed at 5 and 25 ppm. In addition, increased nasal airflow resistance was observed at all concentrations. No change in closing volume was observed (Study ID ◆ 063).

Islam et al. (1994) exposed 37 healthy, nonsmoking volunteers to 0.73±0.05 ppm of SO_2 or cold air for 5 minutes and observed airway response. A statistically greater increase in SRaw was observed after hyperventilation with SO_2 than after hyperventilation with cold air (Study ID ◆ 318).

Andersen et al. (1977) induced rhinovirus infection in normal adults both with and without previous controlled SO_2 exposure. The group exposed to 5 ppm SO_2 for 4 hours was found to have fewer symptoms of infection than the unexposed group. However, there was no difference in the number of subjects who developed infection between the SO_2-exposed and unexposed groups. The only difference between the two groups was a decrease in nasal mucus flow rate in the anterior parts of the nose in the SO_2-exposed group (Study ID ◆ 048).

Nadel et al. (1965) exposed healthy adults to 4 to 6 ppm SO_2 for 10 minutes. They observed significant decreases in airway conductance and thoracic gas volume after exposure (Study ID ● 069).

In a study by Frank et al. (1964), healthy male adults were exposed to three levels of SO_2: 1-2 ppm, 4-6 ppm, and 14-17 ppm for 30 minutes. No change in pulmonary flow resistance was observed at the lowest exposure. Some increase in pulmonary flow resistance was observed at the middle exposure, and an even greater increase was observed at the highest exposure (Study ID ● 076).

Frank et al. (1961) exposed 11 healthy adult male volunteers to 1, 5, and 13 ppm SO_2 to investigate the effect on pulmonary flow resistance. There seemed to be a dose-response trend with some of the volunteers. Most of the results in this study were not significant and there was substantial variability among the volunteers (Study ID ● 323).

Douglas and Coe (1987) discovered that the threshold concentration of SO_2 required to induce lung effects (1 ppm) is less than that required to induce eye effects (5 ppm) (Study ID ● 121).

Amdur et al. (1953) observed decreased tidal volume and increased respiratory and pulse rates in healthy subjects exposed to 1 to 8 ppm SO_2 for 10 minutes (Study ID ● 032).

Lawther et al. (1975) exposed healthy adults to SO_2 concentrations ranging from 1 to 30 ppm in a variety of experiments involving both deep and shallow breathing for durations of 10 minutes to one hour. Small but significant changes in SRaw were observed following hyperventilation of 1 ppm, for an undetermined period of time. Significant changes in response were observed for most, but not all, of the subjects after "quiet breathing" of 10, 15, 20, and 30 ppm for 10 minutes at each concentration. No significant changes were observed for normal breathing at 1 ppm and were varied for deep breathing at 3 ppm. The numbers of subjects varied for each part of the experiment, exposure method varied for each experiment and exposure durations are not always clearly stated in the paper. Not all of the experiments were blinded, and some of the subjects were smokers (Study ID● 317).

Sim and Pattle (1957) exposed healthy male adults to various aerosol and gas mixtures by mask and in a chamber. Maximum dosages were 2160 mg min/m^3 (mask) and 3620 mg min/m^3 (chamber). No effects were observed at dosages below 800 mg min/m^3. Above 1300 mg min/m^3, resistance to air flow was significantly increased in half the volunteers receiving exposure both by mask and by chamber. At this dosage and higher, high-pitched musical rales were observed with a tendency to prolongation of the expiratory phase of respiration. However, sample size was not given and exposure levels and number of exposures for each volunteer were not clearly stated (Study ID ● 324).

Whittenberger and Frank (1963) exposed work colleagues to 1, 5, and 13 ppm for an unidentified amount of time. The health status of the volunteers is unknown, but was assumed to be healthy, except for one subject with a history of childhood asthma. Increases in airway resistance were observed at 5 and 13 ppm for the group, with the exception of the childhood asthma case, who showed airway resistance at 1 ppm. The reported details of this study were limited and unclear (Study ID ● 416).

No effect observed-healthy subjects

Several studies observed no effects on respiratory function in either healthy or asthmatic subjects as a result of exposure.

Stacy et al. (1981) exposed healthy adult males to 0.75 ppm SO_2 for four hours. Each four-hour exposure session included two 15-minute periods of controlled moderate exercise. Nineteen different pulmonary function measurements were taken just prior to exposure, two and four hours into exposure, following each exercise session during exposure, and 24 hours after the exposure. They observed no statistically significant effects in any of the pulmonary function measurements during or after exposure to SO_2 (Study ID ▲ 043).

Kreisman et al. (1976) evaluated the respiratory function of healthy adults breathing 0.5 to 5 ppm SO_2 by mouth for 1 to 5 minutes. They observed variable airway response to SO_2 exposure and were not able to determine a dose-response relationship with increasing concentration or time of exposure (Study ID ◆ 039).

Bedi et al. (1979, 1982) examined the effect on pulmonary function of exposure to 0.4 ppm SO_2 for two hours in healthy male subjects. They observed no changes in FEV_1 (Study ID ◆ 051, 049).

Burton et al. (1969) observed no significant changes in pulmonary function parameters after exposure to 1.1 to 3.6 ppm for 30 minutes in healthy adults (Study ID ◆ 113). Folinsbee et al. (1985) observed no pulmonary function effects after exposing healthy adults to 1 ppm SO_2 for 2 hours during which time the subjects exercised for three 30-minute periods (Study ID ◆ 122).

Healthy adults were exposed to 2 ppm SO_2 for 30 minutes via free breathing or forced oral or nasal breathing with continuous moderate exercise (Bedi and Horvath, 1989). Pulmonary function was measured before and after exposure. No changes in pulmonary function were observed by any of the three breathing methods (Study ID ◆ 266).

Kulle et al. (1984) exposed healthy adults to 1 ppm SO_2 for four hours per day, three days per week for three weeks. They observed no changes in pulmonary function or bronchial reactivity (Study ID ◆040).

In an effort to standardize procedures for human SO_2 exposure tests, Sandstrom et al. (1988) exposed healthy adults to SO_2 concentrations from 0.4 to 4 ppm for 20 minutes. They observed no changes in pulmonary function parameters as a result of SO_2 exposure (Study ID ● 087).

Lawther (1955) exposed healthy male adults to concentrations of 0, 5, 10, and 20 ppm for 10 minutes each by nose and mouth. No significant consistent changes in respiration were observed in the groups of volunteers. There is limited information on the experimental methods and the reporting of the results is lacking in detail (Study ID ●325).

No effect healthy subjects; effect asthmatics

Several research groups subjected both asthmatic and healthy subjects to the same SO_2 exposure. Some of these studies reported respiratory effects in asthmatics but not in normal subjects.

Jaeger et al. (1979) exposed normal and asthmatic subjects to 0.5 ppm SO_2 for 3 hours and measured pulmonary function parameters. They found no effects from the exposure in the normal subjects; however, they did observe a small decrease in mid-maximal expiratory flow in asthmatics (Study ID ▲ 073).

Schachter et al. (1984) exposed ten asthmatic and ten healthy subjects to 0, 0.25,0.50, 0.75, and 1.0 ppm SO_2 for 40 minutes during exercise. No changes in pulmonary function were observed in the healthy subjects at all concentrations or in asthmatics at concentrations below 1.0 ppm. At 1.0 ppm significant changes in SRaw, FEV_1, and max flow at 50% of vital capacity were observed in the asthmatics (Study ID ◆306).

Linn et al. (1987) exposed 24 normal, 21 atopic, 16 minimal or mildly asthmatic, and 24 moderate or severe asthmatics to 0, 0.2, 0.4, and 0.6 ppm SO_2 for one hour including three 10-minute exercise periods. All the normal and most atopic subjects showed little response at all SO_2 levels. The moderate to severe asthmatics showed the most unfavorable overall responses. In both asthmatic groups, there was a general trend towards increasing response with increased dose. However, responsiveness was variable among individuals and could not be predicted by clinical status (Study ID ◆ 309).

Tan et al. (1982) exposed normal and asthmatic adults to concentrations from 2.5 to 20 ppm SO_2 for 5 minutes. Pulmonary function effects, measured as decreases in specific airway conductance, were observed in both groups, but were significant only in the asthmatic group (Study ID ● 092).

Harries et al. (1981) observed no effect in FEV_1 in non-asthmatics exposed to concentrations of SO_2 up to 15 ppm.

However, asthmatic subjects exhibited decreased FEV_1 at concentrations between 5 and 11.5 ppm. Exposure duration was not clear in the study report (Study ID ● 108).

Effects observed-asthmatic subjects

Gong et al. (1995) investigated the hypothesis that SO_2 induces asthma more than other everyday respiratory stressors. They exposed adult asthmatics to concentrations between 0, 0.5, and 1 ppm SO_2 for 10 minutes, measuring various pulmonary function parameters. They observed adverse changes in pulmonary function at 1 ppm. Exercise exacerbated the effect of SO_2 (Study ID ▲ 077).

Roger et al. (1985) exposed adult asthmatics to 0, 0.25, 0.5, and 1 ppm SO_2 for 75-minute periods during which the subjects did three 10-minute periods of moderate exercise. They observed a dose-dependent increase in specific airway resistance at the 0.5 and 1 ppm levels; however, no effects were observed at 0.25 ppm. They also observed that increases in specific airway resistance after the second and third exercise periods were significantly less than after the first exercise period (Study ID ▲ 081).

Jorres and Magnussen (1990) exposed asthmatic adults to concentrations of 0.5 ppm for 30 minutes of tidal breathing followed by 0.75 ppm during hyperventilation. They observed a small but significant change in SRaw after the exposure (Study ID ▲ 109).

Trenga et al. (1999) found a very diverse response to SO_2 exposure at 0.5 ppm for 10 minutes in adult subjects with mild to moderate asthma. Just over half of the subjects experienced a decrease in FEV_1 and changes in peak expiratory flow (Study ID ◆ 055).

Balmes et al. (1987) investigated the relationship between duration or concentration of exposure to SO_2 and bronchoconstriction in asthmatics. They exposed asthmatics to 0.5 or 1 ppm SO_2 for 1, 3, and 5 minutes through a mouthpiece during eucapnic hyperpnea. They observed small increases in specific airway resistance at both concentrations for 1 minute of exposure. Most subjects developed wheezing, chest tightness or dyspnea after inhalation of 0.5 ppm for both 3 and 5 minutes and 1 ppm for 3 minutes (Study ID ◆ 064).

Tunnicliffe et al. (2001) exposed asthmatic and healthy adults to 200 ppb SO_2 for 1 hour. They observed a small, significant increase in mean respiratory frequency in the asthmatics. Other respiratory measures did not differ between the two groups (Study ID ◆ 071).

Sheppard et al. (1980) investigated whether subjects with mild asthma or seasonal rhinitis have a greater bronchomotor response to SO_2 than normal subjects during mouth breathing. They exposed adult volunteers to 1, 3, and 5 ppm SO_2 for 10 minutes each. Increases in SRaw occurred at lower concentrations in mild asthmatics (4 of 7 asthmatics and 0 of 7 normals at 1 ppm). At 5 ppm all of the asthmatics and 5 of 7 normals exhibited significant increases in SRaw (Study ID ◆375).

Tam et al. (1988) investigated whether bronchomotor responsiveness to SO_2 exposure is related to increased nasal responsiveness. Subjects with asthma or chronic rhinitis were exposed to 2 ppm SO_2 for 4 minutes or 4 ppm SO_2 for 10 minutes (Tam et al., 1988). Significant changes were observed in SRaw and lower airway symptoms after breathing SO_2 compared to breathing room air. Significant changes in nasal symptoms or nasal resistance were not observed (Study ID ◆ 062).

Kehrl et al. (1987) exposed adult asthmatic subjects to 1 ppm SO_2 to test the effect of exercise and SO_2 exposure. They observed an attenuated response to repetitive exercise, measured by increases in specific airway resistance during three 10-minute exercise periods separated by 15-minute rest intervals. They also observed that pulmonary function effects occur rapidly and are maintained during a 30-minute continuous exercise period (Study ID ◆ 078).

In an investigation of the effects of SO_2 exposure on total respiratory resistance and forced expiratory volume, McManus et al. (1989) exposed asthmatic adults to 0.5 or 1 ppm SO_2 for 20 minutes at rest followed by 10 minutes of moderate exercise. They observed a statistically significant dose-response effect on FEV_1, specific total respiratory resistance, $V_{max\ 50}$ and $V_{max\ 75}$ (Study ID ◆ 098).

Heath et al. (1994) investigated a difference in ethnic susceptibility in asthmatic pulmonary function response when exposed to SO_2 at 1 ppm for 10 minutes at rest and 10 minutes exercising. They observed no ethnic difference, although both groups showed significant decreases in V_{max50}, R_T and FEV_1 compared to pre-exposure values (Study ID ◆ 110).

Bethel et al. (1985) exposed adult asthmatics to 0.25 ppm SO_2 for 5 minutes at rest and 5 minutes during either moderate or heavy exercise. They observed an increase in SRaw during exercise both with and without SO_2 exposure. However, the increase was slightly, but significantly greater with SO_2 exposure than with filtered air (Study ID ◆ 118). Horstman et al. (1986) exposed 27 adults with mild asthma to 0.25, 0.5, 1.0 and 2.0 ppm SO_2 for 10 minutes during moderate exercise with natural breathing. Substantial variability was observed in bronchial sensitivity to SO_2. The concentration of SO_2 which provoked an increase in SRaw 100% greater than the response to clean air ranged between 1.28 and 1.90 for 23 of the subjects, while for the remaining subjects it was greater than 2.00 ppm (Study ID ◆ 303).

Linn et al. (1983a) exposed adult asthmatics to 0.75 ppm SO_2 for 10 minutes during heavy exercise in a chamber, once with unencumbered breathing and once with nose clips and mouthpieces. Greater increases in SRaw were observed upon exposure to SO_2 than with clean air exposure, with the excess increase significantly greater with mouthpiece than with unencumbered breathing (Study ID ◆ 304).

In another study, Linn et al. (1983b) exposed 23 asthmatic adults to 0, 0.2, 0.4, and 0.6 ppm SO_2 for 5 minutes during heavy exercise. There seemed to be a dose-response effect with only the changes at 0.6 ppm being highly significant. The effects seemed to reverse in less than 1 day (Study ID◆ 310).

Horstman et al. (1988) conducted an investigation of the shortest duration of time required to induce bronchoconstriction in adult asthmatics with 1.0 ppm SO_2 during mild exercise. The concluded that significant increases in bronchoconstriction occurred at 2.0 minutes of exposure (Study ID ◆ 311).

Bethel et al. (1983) exposed 9 asthmatic adults to 0.5 ppm SO_2 for 5 minutes while engaging in light, moderate and heavy exercise. Bronchoconstriction was observed during moderate and to a greater degree with heavy exercise when the volunteers breathed through a mouthpiece. Bronchoconstriction was only observed during heavy exercise when the volunteers breathed through a facemask. Results were variable among volunteers (Study ID ◆ 326).

Sheppard et al. (1981b) investigated whether moderate exercise modifies the bronchoconstriction produced by SO_2 in mild asthmatics. They exposed asthmatics to 0.10, 0.25, 0.50, and 1 ppm of SO_2 through a mouthpiece for 5 or 10 minutes during moderate exercise. Significant bronchoconstriction response was observed for most of the group at 0.25 ppm. The two most responsive volunteers responded at 0.10 ppm. The investigators question whether these responses would occur with oronasal breathing (Study ID ◆ 376).

Wolff et al. (1984) subjected steelworkers with respiratory difficulties to controlled SO_2 exposures at 5 ppm for 2.5 hours. They observed a significant increase in bronchial reactivity after SO_2 exposure. However, changes in actual pulmonary function were seen in only two of the nine subjects (Study ID ● 084).

Fine et al. (1987) observed an increase in SRaw in asthmatics after eucapnic hyperpnea of SO_2 at concentrations up to 8 ppm for one minute (Study ID ● 116).

Linn et al. (1984c) exposed asthmatic adults to 0.6 ppm SO_2 for 6-hour periods on two successive days. The volunteers exercised heavily for 5 minutes at the beginning of the exposures and after 5 hours of exposure. Substantial bronchoconstrictive responses were observed only immediately after exercise. These responses were moderately less severe on the second day of exposure compared to the first (Study ID ● 316).

No effect observed-asthmatic subjects

Bailey et al. (1982) exposed asthmatic subjects to 0, 0.25, and 0.5 ppm SO_2 for one hour by mouthpiece, alternating rest periods with 10-minute periods of moderate exercise. They observed no significant effects in pulmonary function parameters (Study ID ▲ 075). Devalia et al. (1994) exposed asthmatic subjects to 200 ppb SO_2 for 6 hours and measured pulmonary function parameters. They found no significant effects from exposure to SO_2 alone (Study ID ◆ 067).

Linn et al. (1985a) exposed subjects with chronic obstructive pulmonary disease to SO_2 at concentrations up to 0.8 ppm for one hour. They observed no effect on pulmonary function from this exposure (Study ID ◆ 307).

Investigations of effect and recovery

Various studies observed a recovery from symptoms either during or after exposure and after a significant effect on pulmonary function.

Sheppard et al. (1983) investigated whether bronchoconstriction patterns induced by low-level exposures to SO_2 would change upon repeated exposure. They exposed seven non-smoking asthmatics to 0.5 ppm SO_2 for 3 minutes of voluntary eucapnic hyperpnea three times at 30-minute intervals. Seven days later, the same subjects were given only one three-minute exposure. There was a significantly greater increase in specific airway resistance in the first exposure than the second or third exposures. After seven days, specific airway resistance increased as much as it had after the very first SO_2 exposure. The researchers concluded that repeated low-level exposures can induce temporary bronchomotor tolerance to SO_2 in asthmatic subjects (Study ID ◆ 061).

Hackney et al. (1984) investigated the reversibility of bronchomotor effects from SO_2 exposure. Adult asthmatics were exposed to 0.75 ppm SO_2 for three hours, during which the subjects exercised vigorously for the first 10 minutes and rested for the balance of the exposure time. Specific airway resistance was significantly increased after exercise. However, these changes were no longer significant after one hour of exposure. These increases did not persist longer than two hours in the majority of the subjects (Study ID ◆ 079).

Linn et al. (1998) exposed adult asthmatics to 0, 0.3 and 0.6 ppm SO_2 for 10 minutes during heavy exercise. Exercise-induced bronchospasm was observed with no SO_2 exposure. However, bronchoconstriction increased as SO_2 exposure concentration increased. By 30 minutes post-exposure, the lung function of most subjects had returned to pre-exposure levels (Study ID ◆ 097).

Gokemeijer et al. (1973) exposed adults with chronic non-specific lung disease to 10 ppm SO_2 for 3 minutes and observed a marked bronchial obstruction at the end of exposure that decreased to pre-exposure levels 45-60 minutes post-exposure (Study ID ◆ 260).

Toyama and Nakamura (1964) examined changes in pulmonary airway resistance after inhalation of SO_2 at concentrations of 1 to 60 ppm for five minutes in healthy male adults. They observed an increase in pulmonary airway resistance during exposure at all levels, which disappeared several minutes into the exposure. By 15 to 20 minutes post-exposure, airway resistance had returned to control values (Study ID ● 053).

Nose vs. mouth exposure

Several research groups investigated differences in effect as a result of nasal *vs.* oral inhalation. Nasal breathing seems to be more relevant during normal breathing in the absence of physical activity whereas mouth breathing is more relevant during periods of exercise. Speizer and Frank (1966b) exposed healthy male subjects to 15 or 28 ppm SO_2 by inhalation either through the nose or the mouth for 10 minutes. They observed increases in pulmonary flow resistance from both nasal and oral exposure. However, there were more and greater responses from oral than nasal inhalation. There was also greater response from exposure to the higher concentration, on average (Study ID ◆ 054). Kirkpatrick et al. (1982) investigated the effect of breathing route on the bronchomotor response of asthmatics to SO_2 exposure. Asthmatic adults were exposed to humidified air with 0.5 ppm SO_2 for 5 minutes during light to moderate exercise either by mouthpiece (oral breathing), by facemask (oronasal breathing) or by facemask with mouth occluded (nasal breathing). SRaw was increased with all exposure routes. There was no difference in SRaw increases between the oronasal exposure and the oral exposure. However, there was significantly greater increase in SRaw with these two exposure methods than with the nasal exposure. They concluded that nasal breathing seems to provide some protection against SO_2-induced bronchoconstriction in asthmatics exposed to low concentrations of SO_2 (Study ID ◆ 074).

Melville (1970) exposed healthy adults to concentrations of SO_2 between 2.5 and 10 ppm for 10 minutes to an hour by both oral and nasal exposure routes. A statistically significant decrease in specific airway conductance was observed with both exposure routes; however, the effect was greater with oral exposure than with nasal exposure (Study ID ◆ 105).

Bedi and Horvath (1989) observed no significant differences in ventilatory parameters between free-breathing exposure to 2 ppm SO_2 or filtered air in healthy subjects for 30 minutes. However, they did observe significant difference between the free-breathing and the forced oral SO_2 exposure (Study ID ◆ 266).

Effects of temperature and humidity

Sheppard et al. (1984) examined the combined effect of dry (cold) air and SO_2 inhalation on pulmonary function in asthmatic adults. They observed bronchoconstriction at significantly lower SO_2 concentrations when the SO_2 was inhaled in dry cold or warm air rather than humidified warm air. Bronchoconstriction was observed with concentrations in dry air as low as 0.1 ppm for 3 minutes (Study ID ◆ 057).

Bethel et al. (1984) investigated the interaction of cold dry air and SO_2 on asthmatic subjects. Asthmatic and healthy adults were exposure to 0.5 ppm SO_2 in humidified room-temperature air and cold dry air for 3 minutes each. No effects were observed with the humidified room-temperature air with SO_2 for either set of subjects; however, there was an increase in SRaw with exposure to the cold dry air with SO_2 in the asthmatic subjects but not the healthy subjects (Study ID ◆ 123).

Linn et al. (1984a) exposed 24 adult asthmatics to 0, 0.3, and 0.6 ppm SO_2 for 5 minutes at each of three temperatures: 21°C, 7°C, and –6°C with constant relative humidity of approximately 80%. While there was considerable variability between the subjects, cold seemed to exacerbate the overall response to SO_2. The combined stresses acted additively at most, but not synergistically (Study ID ◆ 314).

In a similar study, Linn et al. (1984b) exposed 8 adult asthmatics to 0, 0.2, 0.4, and 0.4 ppm SO_2 for 5 minutes during heavy exercise with both high (85%) and low (50%) relative humidity. Bronchoconstriction increased with increasing SO_2 concentrations, but did not vary significantly with humidity. However, these results should be interpreted with caution, given the small sample size and limitations in experimental design. In another experiment in the same study, 24 adult asthmatics were exposed to 0.6 ppm SO_2 at 5°C and 22°C at high relative humidity. No significant changes in response were observed between the two temperature conditions (Study ID ●313).

Further investigating the effects of temperature and humidity on SO_2 reactivity in asthmatics, Linn et al. (1985a) exposed 22 adult asthmatics to 0.6 ppm SO_2 for 5 minutes during heavy exercise at temperatures of 21°C and 38°C and a relative humidity of 20% and 80%. Greater effects on SRaw were observed at low temperature and low humidity (Study ID ◆ 307).

Non-clinical studies

Effects observed-bronchial clearance

Ferin and Leach (1973) investigated the effect of SO_2 on the clearance of inert particles (TiO_2) from rat lungs. At exposure to 1 ppm for 170 hours, a slight but statistically significant change in lung clearance was observed. Shorter exposures to 20 ppm also resulted in a statistically significant decrease in clearance (Study ID ◆ 235).

In investigating the effect of SO_2 exposure on early and late clearance of inhaled soluble tracer particles, Mannix et al. (1983) exposed rats to 20 ppm SO_2 for 4 hours. Early clearance (upper respiratory tract) was significantly delayed as a result of SO_2 exposure compared to controls, while late clearance (deep-lung) rates were not significantly different from controls (Study ID ◆ 256).

Oomichi and Kita (1974) investigated the effect of SO_2 exposure on ciliary clearance in excised guinea pig tracheae. A dose-dependent decrease in ciliary activity was observed at exposure to 15, 32, 58, and 77 ppm for 2 to 6 minutes (Study ID ◆ 213).

Riechelmann et al. (1995) examined changes in mucociliary activity in guinea pig trachea with exposure to SO_2 concentrations of 3, 6, 9, 11, and 14 ppm for 30 minutes. They observed a dose-dependent reduction in mucociliary activity (Study ID ● 132).

Knorst et al. (1994) also investigated the effect of SO_2 exposure on mucociliary activity and ciliary beat frequency on guinea pig tracheas. They exposed tracheal samples to 2.5, 5, 7.5, 10, and 12.5 ppm SO_2 for 30 minutes and observed a statistically significant decrease in mucociliary activity at 2.5 ppm. A dose-dependent decrease in ciliary beat frequency was observed at SO_2 concentrations higher than 5 ppm, in addition to further reductions in mucociliary activity at higher SO_2 concentrations (Study ID ● 164).

Trimpe et al. (1986) investigated the effect of SO_2 on the clearance of *Listeria monocytogenes* from normal and emphysematous hamster lungs. After exposure to 27±3 ppm SO_2 for 35 days, a decrease in the number of *L. monocytogenes* recovered from both normal and emphysematous hamsters was observed (Study ID ● 134).

A number of studies investigated the nasal mucociliary transport rates in chickens after exposure to various SO_2 concentrations and durations of exposure. Wakabayashi et al. (1977) exposed chickens to SO_2 intermittently for 16 hours a day for 7 days at concentrations of 1.4 to 66 ppm. The mucociliary transport in the nasal mucus membranes was observed. Peaks of increased intranasal transport time with intervening recovery periods were observed at all concentrations. Transport in the sinus was decreased at concentrations above 10 ppm (Study ID ● 129).

Ukai et al. (1984) also investigated mucociliary function in chickens. After exposure to concentrations between 18 and 40 ppm, a deceleration of turbinate clearance was observed, as was a decrease in sinus clearance rates (Study ID ● 137). A previous study (Ukai et al., 1983) found similar decreases in turbinate clearance in chickens for both continuous and intermittent SO_2 exposure for 1 hour, 4 times/day for 2 days at concentrations between 4 and 40 ppm (Study ID ● 138). Majima et al. (1985) also observed decreases in mucociliary trânsport rate in chickens exposed to 6 ppm SO_2 16 hours a day for 7 days (Study ID ● 149).

Effects observed-bronchoconstriction or specific airway resistance

Chickens

No effects on tidal volume or respiratory frequency were observed in chickens exposed to 100 ppm SO_2 breathing through their nostrils and mouth, but a small, statistically significant increase in minute volume was observed (Fedde and Kuhlman, 1978). At 500 ppm SRaw decreased; at 1000 ppm SRaw initially decreased, then subsequently increased. Also at 1000 ppm, respiratory frequency and minute volume decreased. The effects seen at 1000 ppm were increased at 5000 ppm. All exposures lasted 60 minutes (Study ID ▲ 183).

Rabbits

Barthelmy et al. (1988) investigated cold-induced bronchospasm in rabbits exposed to 0.5 or 5.0 ppm SO_2 for 45 minutes. Dose-dependent increases in lung resistance were observed, returning to control values by 40 minutes post-exposure. However, cold-induced bronchoconstriction was decreased by prior exposure to SO_2, suggesting a protective effect on SO_2 exposure and cold-induced bronchomotor response (Study ID ▲ 197).

Davies et al. (1978b) exposed rabbits to either 300 ppm for three hours or 150 ppm for 12 three-hour periods. They observed higher lung resistance in animals exposed to 300 ppm, three days after exposure, but no differences in those animals exposed to 150 ppm. In addition, both groups of animals exhibited decreased breathing frequency, but recovery times were faster for those animals exposed to 300 ppm for three hours (Study ID ◆ 239).

Davenport et al. (1984) exposed rabbits to 200 to 400 ppm SO_2 for 15 to 20 minutes. They observed decreased breathing frequency and increased tidal volume in the exposed animals compared to controls (Study ID ◆ 244).

Citterio et al. (1985b) exposed rabbits to 300 to 350 ppm for an unreported total exposure time. Statistically significant increases in inspiratory and expiratory time were observed as well as a rising rate of diaphragm activity. The effects were observed for up to 30 minutes post-exposure (Study ID ● 194).

Davies et al. (1978a) observed decreased Breuer-Hering reflex and activity in 23 of 26 stretch receptors as well as increased inspiratory time and decreased expiratory time in rabbits exposed to 200 ppm SO_2 for 10-minute periods (Study ID ● 234).

Guinea pigs

Park et al. (2001) investigated enhanced pause as an index of airway obstruction as a result of SO_2 exposure. Guinea pigs were exposed to 0.1 ppm SO_2 for five hours a day for five days. Significant increases in respiratory pause were observed after SO_2 exposure (Study ID ▲ 259).

Guinea pigs exposed to levels of 50 to 500 ppm SO_2 for 15 minutes exhibited reductions in dynamic compliance and conductance (Atzori et al., 1992). No effects were seen at lower concentrations; however, pretreatment with 10 ppm provided a protective effect against bronchoconstriction upon subsequent exposure to 250 ppm SO_2 (Study ID ◆ 189).

Amdur et al. (1983) investigated the effect SO_2 and ZnO alone and in combination on the respiratory mechanics of guinea pigs. 1 ppm SO_2 for one hour resulted in an increase in respiratory resistance and a decrease in respiratory compliance, both statistically significant. Mixtures of ZnO and SO_2 resulted in changes in lung function relative to control animals that were not always significantly different from SO_2 exposure alone (Study ID ◆ 229). Halinen et al. (2000a) exposed guinea pigs to SO_2 in cold dry air for four consecutive 10-minute periods at concentrations of 0, 1, 2.5, and 5 ppm. A dose-dependent increase in bronchoconstriction was observed at 1 and 2.5 ppm compared to the initial exposure to clean dry air. In the fourth exposure period, which involved concentrations of 5 ppm, a smaller bronchoconstriction response was observed (Study ID ◆ 245). In a follow-up study, guinea pigs were exposed to 1 ppm SO_2 in cold dry air for one hour continuously (Halinen et al., 2000b). Weaker effects on lower respiratory function were observed in this study than in the first study (Study ID ◆ 246). Amdur (1959) exposed guinea pigs to SO_2 concentrations between 2 and 1000 ppm. A dose-dependent increase in bronchial constriction was observed after a one-hour exposure at these levels. Bronchoconstriction was increased for the duration of a three-hour exposure to 24 ppm, with lung function returning to baseline values within three hours post-exposure. Bronchial constriction was greater when animals were exposed through tracheal cannulae rather than respiring normally (Study ID ● 216). Amdur and Underhill (1970) exposed guinea pigs to SO_2 and Fe_2O_3 or open-hearth dust, singly and in combination, to determine any interaction effects. They observed significantly increased levels of airway resistance upon exposure to SO_2 levels from 1.5 to 26 ppm for one and two hours. The combination of pollutants did not result in significantly different levels of respiratory response from SO_2 exposure alone (Study ID ● 227).

Mice

Sensory irritation, as measured by decreased respiratory rate, was investigated in mice exposed to SO_2 (Alarie et al., 1973). Single exposures to 17, 32, 62, 89, 123, 198, and 298 ppm for 10 minutes decreased respiratory rate significantly compared to no exposure. A dose-dependent relationship was observed between rapidity of onset and depth of respiratory depression, and SO_2 exposure (Study ID ◆ 243). Leong and MacFarland (1965) exposed rats to SO_2 concentrations of 40, 64, 83, 145, 231, 426, and 751 ppm for two hours to observe indications of respiratory stress. They found a dose-dependent decrease in percent SO_2 retention, respiratory rate, and minute volume as SO_2 concentration increased (Study ID ◆ 253).

Dogs

Cho et al. (1968) exposed anaesthetized dogs to 11 to 1000 ppm SO_2 for 0.1 to 6 minutes. Exposure at all levels initiated bronchoconstriction in all the dogs tested (Study ID ● 167). Frank et al. (1965) exposed mongrel dogs to SO_2 concentrations ranging between 7 and 230 ppm for 15 to 20 minutes. Exposures were by nose, by tracheal cannula, and by an isolated segment of trachea. Exposure by nose resulted in an observed increased in nasal flow resistance (Rn), roughly proportional to SO_2 concentration. Rn reverted partially or totally to control levels within 15 to 40 minutes post-exposure. Pulmonary flow resistance (Rl) followed the same pattern, with smaller effects observed, and an increase in Rl during recovery. The greatest change in Rl was observed with tracheal cannula exposure. A lesser increase in Rl was observed with isolated tracheal exposure (Study ID ● 170).

Lewis and Kirchner (1984) exposed dogs to 10 and 30 ppm SO_2 for 5 minutes. Pulmonary resistance and compliance did not change at 10 ppm. However, increased lung hypersensitivity to aerosolized methacholine occurred after exposure to 30 ppm and was maximal at four hours post-exposure (Study ID ● 258).

Eady and Jackson (1989) exposed dogs to 400 ppm SO_2 for 2 hours. They observed an immediate increase in bronchial response to histamine. This response returned to normal levels by 2 hours after exposure. However, 24 hours after exposure a second phase of bronchial responsiveness occurred which lasted for several days (Study ID ● 190).

Islam et al. (1972) exposed dogs to 0, 1, 2, 5, and 10 ppm SO_2 for three 60-minute periods and observed increased bronchial sensitivity to acetylcholine compared to controls. Maximum sensitization was observed at 2 ppm SO_2 (Study ID ● 162).

Norris and Jackson (1989) reported airway hyperreactivity to histamine in dogs exposed to 200 ppm SO_2 for 2 hours (Study ID ● 146).

Frank and Speizer (1965) exposed dogs to concentrations of 7-16 ppm, 25-34 ppm, or 60-61 ppm SO_2 for 20 minutes by nose, tracheal cannula, or by an isolated segment of the trachea. Nasal flow resistance increased in a roughly dose-dependent manner during nasal exposure. Recovery took between 15 and 40 minutes. Little change was observed in pulmonary flow resistance. During exposure by tracheal cannula, pulmonary flow resistance increased quickly to a peak, after which it receded. The isolated tracheal exposure produced less pronounced changes in pulmonary flow resistance. All of the observed effects were variable (Study ID ● 170).

Cats

Grunstein et al. (1977) exposed cats to 3000 to 7000 ppm SO_2 for 24 to 40 seconds. They observed a reduction in tidal volume, and increased respiratory frequency and pulmonary resistance (Study ID ● 186).

Corn et al. (1972) exposed 20 healthy male cats to 15-25 or 30-40 ppm SO_2 for 30 minutes and observed alterations in pulmonary flow resistance. No animals responded at concentrations lower than 20 ppm and only one animal showed a significant increase in pulmonary flow resistance at this level. Results were variable between the cats. There are inconsistencies in the reporting and limited detail in the experimental methods and results sections of the paper (Study ID ●290).

Thompson et al. (1990) exposed cats to 100, 500, 800, and 1000 ppm SO_2 for 1, 5, and 10 breaths. A concentration dependent response was observed in lung resistance with the administration of 10 breaths of 100 to 1000 ppm SO_2. The results were variable among the subjects. Limited information on the experimental design and the results is provided. The study design did not appear to follow Good Laboratory Practice guidelines (Study ID ●372).

Sheep

Allergic and normal sheep were exposed to 5 ppm SO_2 for 4 hours to investigate airway reactivity (Abraham et al., 1981). No differences from control values were observed in either normal or allergic sheep directly after exposure. 24-hours after exposure, airway reactivity in the allergic sheep increased significantly (Study ID ◆ 230).

A similar study exposed normal and allergic sheep to SO_2 for 4 hours (Abraham et al., 1980). In this study normal sheep were divided into two groups based on SO_2 exposure concentrations: 5 and 10 ppm. The allergic sheep were exposed to only 5 ppm. Airway reactivity was not significantly changed directly after exposure; however, both the normal sheep exposed to 10 ppm SO_2 and the allergic sheep exhibited increased airway reactivity 24-hours post exposure (Study ID ◆ 231).

Donkeys

Spiegelman et al. (1968) exposed miniature donkeys to SO_2 levels ranging from 27 to 713 ppm for 30 minutes to observe any effects on bronchial clearance of radioactive monodisperse ferric oxide particles. No effect on bronchial clearance was observed at concentrations below 300 ppm. Higher concentrations produced severe cough and slowing or transient arrest of bronchial clearance (Study ID ● 205).

Effects observed-other

Giddens and Fairchild (1972) exposed mice to 10 ppm SO_2 for 4 to 72 hours to observe the effect of SO_2 exposure on the nasal and respiratory tracts. Lesions consisting of edema, necrosis, and desquamation of the olfactory and respiratory epithelium were observed at 24-hour and longer exposures. More injury was observed in the nasomaxillary turbinates than in the rest of the respiratory tract (Study ID ◆ 191).

Ukai (1977) investigated the effect of SO_2 exposure (0.03 to 0.1 ppm for 4 weeks) on upper respiratory infection response in mice inoculated with influenza virus. More rapid and more severe inflammatory response, as well as more rapid development and higher levels of HI titer were observed in the SO_2-exposed mice (Study ID ◆ 207).

Fairchild (1977) observed an inhibition in the growth of influenza virus in the noses of mice exposed to 6 ppm of SO_2 for 7 days. Virus propagation was not altered (Study ID ◆ 238).

Hanacek (1987) investigated the effect of SO_2 on cough and expiratory reflexes of 22 anaesthetized rabbits. Exposure to 200-300 ppm SO_2 for 10-15 minutes resulted in decreases in both mechanically stimulated cough excitability and cough reflex strength in rabbits. Reporting of the experimental methods and results lack detail (Study ID ●300).

No effects observed

Exposure of guinea pigs to 0.2, 0.4, and 0.8 ppm SO_2 for 2 hours produced no statistically significant changes in respiration (Amdur et al., 1978; Study ID ◆ 204).

Guinea pigs were exposed to 1 ppm SO_2 for two 60-minute exposures at high and low relative humidities (McJilton et al., 1976). No significant change was observed in pulmonary flow resistance with SO_2 exposure at either high or low relative humidity. The objective of the study was to measure the interaction of SO_2 and sodium chloride aerosol. Significant changes in pulmonary function were observed only when the two inhalants were administered together at high relative humidity (Study ID ◆ 257).

Amdur and Underhill (1968) investigated the respiratory response of various soluble and insoluble aerosols, including SO_2, in guinea pigs. No statistically significant changes in pulmonary flow resistance were observed after exposure to 2 ppm SO_2 for 10 minutes (Study ID ● 226). Donkeys were exposed to SO_2 concentrations between 53 and 300 ppm for 30 minutes to investigate the effect of SO_2 exposure on mucus transport rates and the clearance of gamma-tagged insoluble aerosols (Lippman et al., 1975). No changes in mean residence time of the aerosols in the respiratory system were observed at any SO_2 concentrations (Study ID ● 263). Hanacek et al. (1991) observed the cough reflex elicitability (CRE); cough reflex strength (CRS), and Hering-Breuer inflation index (HBIR) in rabbits 24- and 48-hours after exposure to 200 to 300 ppm SO_2 for 10 to 20 minutes. Some changes were observed in the exposed animals but none were significantly different from controls (Study ID ● 161).

Epidemiology studies

Children

Boezen et al. (1999) conducted a study investigating whether children with bronchial hyperresponsiveness and high serum concentrations of total IgE are susceptible to air pollution. They observed a significant increase in the prevalence of lower respiratory symptoms in children with bronchial hyperreactivity and high serum IgE concentrations with 15 ppb incremental increases in ambient air pollution (24 hr mean: 3.2-8.6 ppb). The ORs ranged from 1.28 to 2.49 (95% CI range 1.00-4.04) for lag days 0,1, and 2 and a 5-day mean (Study ID ◆ 005).

Dockery et al. (1982) measured the pulmonary function of children in Steubenville, Ohio, before and immediately after air pollution alerts with SO_2 concentrations between 64 and 174 ppb at various times over a two-year period. Pulmonary function was also measured weekly for three weeks after the alerts. Pulmonary function and SO_2 concentration were analyzed by regression. There was a slight but statistically significant decrease in pulmonary function with increasing SO_2 concentrations (Study ID ◆ 013).

Hoek and Brunekreef (1993) investigated the effect of winter air pollution episodes on the respiratory health of children. Spirometry tests were performed and ambient air concentrations of SO_2, black smoke, PM_{10}, and NO_2 were measured. During an air pollution episode with daily average SO_2 concentrations above 38 ppb, FVC, FEV_1 and MMFR were lower than baseline values. No association was observed between other pollutants and lung function test results (Study ID ◆ 018).

Schwartz et al. (1994) investigated ambient air pollution exposures and respiratory illness in elementary school children in 6 US cities. SO_2 was not significantly associated with cough incidence or upper respiratory symptoms. SO_2 seemed to be significantly associated with lower respiratory symptoms (OR 1.28; (5% CI 1.13-1.46) with 10 ppb incremental increases from an ambient concentration greater than 22 ppb, although this association appeared to be derived from a few influential observations and needs to be interpreted with caution (Study ID ◆ 426).

Segala et al. (1998) found a significant increase in incidence of asthma attack in mild asthmatics with an incremental increase of 19 ppb SO_2 for the same day (OR 2.86, 95%CI 1.31-6.27) and for lag day 1 (OR 2.45; 95%CI 1.01-5.92) while investigating childhood asthma and air pollution in Paris. SO_2 concentrations ranged from 1.7 to 32 ppb with a mean of 8.3±5.1 ppb (Study ID ◆ 448).

Roemer et al. (1993) investigated air pollution and occurrence of respiratory symptoms in children with chronic respiratory symptoms in The Netherlands. A small but statistically significant association (OR not reported) was observed between SO_2 concentrations and both morning and evening peak flow. Highest 24-hour average and 1-hour maximum were 40 ppb and 56 ppb, respectively (Study ID ◆ 449).

Agocs et al. (1997) conducted a longitudinal study of lung peak expiratory flow rates in asthmatic children and ambient air pollution in Budapest, Hungary. A consistent, significant association between SO_2 exposure (Median: 16 ppb Range: 11-55 ppb)and peak expiratory flow was not observed. A training effect was not considered and accurate information on medication use was not available. Exposure to environmental tobacco smoke was common and not accounted for (Study ID ● 362).

Romieu et al. (1995) observed an association between total number of emergency visits for respiratory disease in children and incremental 19 ppb increases in levels of SO_2 on the same day in Mexico City (mean concentrations: 70 ppb; range: 10-490 ppb). The associations between number of emergency visits for asthma or total number of emergency visits and SO_2 concentration were not statistically significant. Relative risks and confidence intervals were not reported, limited details are available on the exposure assessment, misdiagnosis was possible in the very young children and some results are reported to be significant when they are not (Study ID ● 385).

Lin et al. (2003) observed an association between asthma hospitalization and exposure to SO_2 lagged over 6 or seven days in girls aged six to twelve, but not in boys, in Toronto, Ontario. There was limited information on exposure assessment and monitoring. The bidirectional crossover design of the study makes it difficult to compare to other studies (Study ID ● 394).

Lee et al. (2002) observed a statistically significant association (OR 1.11; 95% CI 1.06-1.17) between hospital admissions for asthma in South Korean children and an incremental 4.4 ppb increase in ambient SO_2 (mean 7.7 ±3.3 ppb). However, there was limited information on exposure monitoring and assessment and the results of some of the models were inconsistent (Study ID ● 398).

Delfino et al. (2003) observed significant associations between bothersome (OR 1.23; 95%CI 1.06-1.41) and more severe (OR 1.36; 95%CI 1.08-1.71) asthma symptoms in Hispanic children in Los Angeles with incremental 3.8 ppb increases in ambient SO_2 (mean 6.5 ppb; range: 1.0-26.1 ppb). However, limitations of this study include small, non-random sample, no validation of peak expiratory flow measurements and asthma symptoms, and inconsistent exposure monitoring (Study ID ● 413).

Chew et al. (1999) report a significant positive correlation (OR 1.80-2.90; 95%CI not reported) between an incremental 7.6 ppb increase in SO_2 levels lagged by 1 or 2 days and daily asthma emergency room visits in children in Singapore in this ecological study (mean concentrations 14.5±8.3 ppb). However, confidence intervals are not reported and there was a substantial amount of missing SO_2 data (Study ID ●456).

Hajat et al. (1999) reported a statistically significant association (5.8%; 95%CI 1.6%-10.2%)between GP consultations for asthma and other lower respiratory diseases in children and an approximately 6.8 ppb change in ambient levels of SO_2 (mean 8.4±3.4 ppb). No significant findings were reported for adults and the elderly. Confidence intervals were wide and there was limited information on exposure monitoring (Study ID ● 469).

Mortimer et al (2001) observed an association between a 2-day moving average lag increase in SO_2 (range between cities ~ 5 – 75 ppb; average: 53 ppb) and morning asthma symptoms in asthmatic children aged 4 to 9 years in the USA (OR 1.48). Some confounding factors, such as exposure to cigarette smoke were not considered. Confidence intervals were not given (Study ID ● 432).

Garty et al. (1998) observed a but not statistically significant positive correlation between emergency room visits for acute asthma attacks in children and ambient mean SO_2 concentrations (range 11-27 ppb). The authors also estimated that approximately 28% of the variance in the number of ER visits was explained by fluctuations in SO_2. However, statistical significance was not calculated and both outcome and exposure misclassification are possible (Study ID ●485).

Peters et al. (1996) reported a weak association between incremental 51 ppb increases in SO_2 and decreases peak expiratory flow in children in East Germany and the Czech Republic (mean concentrations: 27.1-90 ppb). However, these results were not statistically significant. Exposure assessment was a limitation of this study as was the outcome data collection (Study ID ● 435).

Queiros et al. (1990) observed very small significant correlations (r = 0.334 monthly p=0.01; r=0.473 quarterly p=0.07) between monthly and quarterly mean ambient SO_2 concentrations (9.1±3.1 ppb) and asthmatic attacks on children in the Oporto area of Portugal. Limited details are given on SO_2 concentrations, consideration of confounders, grouping of outcome status groups, and confidence intervals (Study ID ●445).

Braun-Fahrlander et al. (1992) found no statistically significant associations between ambient SO_2 levels (range: 11-27 ppb) and respiratory symptoms in children in two cities in Switzerland. Details of the analysis of the SO_2 data are limited (Study ID ● 450).

Henry et al. (1991) found no significant association between daily ambient SO_2 levels (>10.9 ppb) and asthma symptoms in children in two towns in New South Wales, Australia. Confounders were not addressed and exposure assessment is a limitation of this study (Study ID ● 451).

Keiding et al. (1995) found no association between SO_2 levels (daily averages: 0-38 ppb) and number of total contacts or contacts for respiratory illness with the Copenhagen Emergency Medical Service in children. An influenza epidemic at the beginning of the study may have confounded the results. In addition, monitoring was a limitation due to the placement of the monitors and lack of detail regarding the variations in SO_2 levels among the monitoring stations (Study ID ● 457).

Yu et al. (2000) observed no significant association between incremental 10 ppb increases in ambient SO_2 concentrations (daily mean: 8.0 ppb) and asthma symptoms in children in Seattle, Washington. Potential confounders such as other outdoor pollutants, meteorological factors, and respiratory infection were not considered and exposure assessment was based on averages of regional monitoring (Study ID ● 462).

Roemer et al. (1998) reported no clear association between SO_2 (daily concentration range: 1.0 – 43.5 ppb) and morning or evening PEF in the Pollution Effects on Asthmatic children in Europe (PEACE) study. Respiratory infections were not considered and may have confounded the analysis. Timing of morning PEF measurements followed a long period of indoor exposure, so exposure assessment using ambient measurements may lead to misclassification (Study ID ● 467).

Hospital admissions or other incidence of chronic obstructive pulmonary disease (COPD)

Dab et al. (1996) examined hospital admissions for respiratory diseases in Paris as part of the APHEA project. 24 hour (mean: 11 ppb) and 1 hour maximum (mean: 23 ppb) SO_2 concentrations were significantly associated with admission for COPD for same-day exposure (Study ID ◆351).

Anderson et al (1997) investigated the short-term effects of air pollution on hospital admissions for COPD in Europe as part of the APHEA project (Air Pollution and Health, a European Approach). The effect of an incremental 19 ppb increase in SO_2 varied considerably across the cities (Amsterdam, Barcelona, Paris, Rotterdam) and was not statistically significant for all ages. In the warm season, borderline significant results (daily OR 1.05 95%CI 1.01-1.10) were observed between hospital admissions for COPD and an incremental 19 ppb increase in daily mean SO_2 levels (17.9-31.3 ppb) with inconsistent lags of either the same day or day 2 (Study ID ◆ 369).

Desqueyroux et al. (2002 a,b) observed no association between physician-monitored exacerbation of COPD symptoms (Study ID ◆ 406) or asthma (Study ID ◆ 402) and mean 24 hr concentrations of SO_2 in Paris. SO_2 concentrations ranged from 0.76 to 10 ppb in the summer (mea: 2.7 ±1.9 ppb) and from 1.1 to 31 ppb in the winter (mean: 7.3±4.6 ppb). ORs were calculated for a 4 ppb increase in SO_2 concentrations.

Tenias et al. (2002) investigated the short-term effects of air pollution on emergency room visits for COPD in Valencia, Spain. An incremental 4 ppb increase of SO_2 was not associated with emergency room visits for COPD (24-hr mean 10.4 ppb; range 2.0-26.1 ppb) (Study ID ◆ 431).

Sunyer et al. (1991) found a small but statistically significant association (0.70/day at 38 ppb $p<0.01$; 0.55/day at 57 ppb $p<0.01$; 0.70/day at 27 ppb $p=0.04$) between daily number of emergency room admissions for chronic obstructive pulmonary disease and daily levels of SO_2 in an ecological study in Barcelona. An incremental increase of 38 ppb daily mean SO_2 levels (24-hr averages: 27, 38, and 57 ppb) led to an average of 2 additional admissions per day. There is some question as to the diagnosis of COPD, suggesting possible misclassification or overestimation of admissions. 20% of the SO_2 monitoring data was unavailable due to malfunctioning equipment (Study ID ● 439). In a later study, Sunyer et al. (1993) concluded that an incremental 9.5 ppb increase in ambient SO_2 levels (baseline unreported) resulted in a 6% increase in COPD emergency admissions in the winter and 9% in the summer in Barcelona over a 5-year period. Socioeconomic confounders could not be considered because outcome data was obtained from registries rather than personal testing or questionnaires. Mean levels of SO_2 and confidence intervals were not reported in this study (Study ID ● 437).

Wong et al. (1999) reported weakly significant associations (≥65 years OR 1.023 95%CI 1.012-1.036; all ages OR 1.013 95%CI 1.004-1.021) between an incremental increase of 4 ppb in ambient SO_2 levels (mean 6.5 ppb; range 1.0-26.1 ppb) and hospital admissions for respiratory disease and COPD. Information on the exposure monitoring is limited and it is suggested by the study authors that exposure classification is a weakness of the study (Study ID ● 481).

Hospital admissions or clinic visits for respiratory disease and/or asthma

Bates and Sizto (1987) investigated hospital admissions and air pollution data in Southern Ontario for January, February, July and August in 1974 and 1976 to 1983. Mean hourly SO_2 peaks were 2.21 to 5.14 ppb in the winter and 1.65 to 3.97 in the summer. Significant correlations (correlation and confidence intervals not reported) were found between SO_2 and deviations from the mean respiratory admissions for day of the week, season and year. The study authors warn that hospital and emergency room admission data may be unreliable in classifying outcomes (Study ID ◆367).

Walters et al. (1994) looked at hospital admissions for respiratory disease and asthma and ambient levels of SO_2 and smoke in Birmingham, UK. Mean and max levels of SO_2 were 15 ppb and 48 ppb, respectively. Daily SO_2 levels were weakly, but significantly (15.5 admissions 95%CI 6-25), associated with hospital admissions for respiratory diseases for the same day in the summer and with a two-day lag in the winter (Study ID ◆ 340).

Wong et al. (2002) looked at daily air pollution and daily hospital admissions for asthma and other respiratory admissions in Hong Kong and London, England. Asthma admissions were not significantly associated with an incremental 4 ppb increase in SO_2 in either city (mean 6.8±4.7 ppb). The association with respiratory admissions was small, but significant in Hong Kong (associations and confidence intervals not reported) (Study ID ◆ 423).

Emerson (1973) studied 32 volunteers with chronic airways obstruction weekly for up to 82 weeks to assess a relationship between respiratory function and changes in atmospheric conditions and air pollution. SO_2 (mean 73.4±40 ppb) was reported to be significantly correlated (associations and confidence intervals not reported) with FEV_1 in one volunteer and with MEFR in two volunteers. The observational protocol varied considerably among the volunteers and significance was not clearly established (Study ID ●342).

Ponce de Leon et al. (1996) investigated short-term effects of London air pollution (daily SO_2 averages: Daily average: 12±5 ppb on hospital admissions for respiratory disease from 1987 to 1992. Weak and questionably significant associations were reported between an increase in SO_2

concentrations from the 10th to the 90th percentile and two different ages groups and two seasons. The significance of these results is questionable due to the number of significant figures used in the calculation of the RR and the confidence intervals (15-64 year group in the cool season for a 7-19 ppb increase in SO_2: RR = 1.0389, 95% CI 1.0010-1.0783; 0-14y group in the warm season for a 7-16 ppb increase in SO_2 RR=1.0468, 95%CI 1.0066-1.0885) (Study ID ● 346).

Ponka and Virtanen (1996b) observed inconsistencies in their observations of associations between ambient air pollution and hospital admissions for asthma. Positive associations (associations not reported) were observed for 24-hour SO_2 concentrations (means: 5-10 ppb) and asthma admissions. However, significant positive associations (associations not reported) were also observed for digestive tract disease (the control) and the control exposure level. This suggests that the modeling may have been unsatisfactory (Study ID ●347).

Hwang and Chan (2002) observed significant associations between current day SO_2 concentrations and daily numbers of clinic visits for lower respiratory illness in Taiwan. SO_2 concentrations ranged from 1.5 to 16.9 ppb with a mean of 5.4±3.0 ppb. People over 65 seemed to be the most susceptible and the associations decreased as lag day increased. Associations and confidence intervals are not reported and the exposure assessment is a limitation (Study ID ●393).

Martins et al. (2002) reported a significant association (18% increase in visits; range 4.14%-31.85%) between a six-day moving average of SO_2 (4.5 ppb) and emergency room visits for chronic lower respiratory disease in the elderly in this ecological study in Sao Paulo, Brazil. There is limited information on exposure monitoring or on the justification of representative cases (Study ID ● 399).

Jaffe et al. (2003) found inconsistent results in Cincinnati, Cleveland, and Columbus, Ohio when investigating the number of daily emergency department visits and air pollution for asthmatics aged 5 to 34 years. The authors report a 12% increased risk of an emergency department episode (95%CI 1-23%) with a 19 ppb incremental increase in SO_2 across all cities (daily mean 13.7±9.6 ppb; 15.0±9.7 ppb; 4.2±3.2 ppb). However, inconsistencies in the reporting of the results, uncontrolled confounding, and potential exposure misclassification limit confidence in these findings (Study ID ● 409).

Hajat et al. (2002) observed seasonally-dependent significant increases in the number of physician consultations for upper respiratory disease in London, England for adults (4.6% change in admissions 95%CI 1.5-7.7%) and those 14 years and younger (cool season: 5.5 % 95%CI 2.4-8.7%; all year: 3.5% 95%CI 1.4-5.8%) associated within incremental increases of SO_2 between 5.7 ppb and 7.8 ppb (mean warm season: 7.8±2.5; mean cool season: 8.4±3.4). Significant changes were not observed in the elderly. The likelihood of both outcome and exposure misclassification limits confidence in this study (Study ID ●410).

Pinter et al. (1996) report significant correlations (associations not reported) between daily concentrations of SO_2 and incidence of acute respiratory morbidity. Confounding factors such as temperature, concentration of pollution from domestic heating (homes heated by low-quality coal), and frequency of infection were not considered and SO_2 levels are not clearly reported (Study ID ● 428).

Hoek and Brunekreef (1994) observed small significant positive associations between previous day SO_2 concentrations (mean: 5.7±5.5 ppb) and daily respiratory symptoms (cough OR 1.10; LRD OR: 1.18), but not pulmonary function in the Netherlands. Pulmonary function testing was carried out but all other symptoms were assessed by diary or interview, introducing the possibility of recall and personal bias. Confidence intervals were not reported (Study ID ● 444).

Schwartz (1995) reported that SO_2 levels were significant predictors of hospital admissions for respiratory disease in two cities in the USA with very different ratios of SO_2-to-PM (SO2 concentrations New Haven 29.8 ppb; Tacoma 16.8 ppb). The lag day differed between the two cities. Climate differences between the cities may have confounded the analysis. Control for O_3 substantially weakened the association with SO_2 and relative risks were very low (New Haven RR 1.03 95%CI 1-1.13); Tacoma RR 1.06 95%CI 1.01-1.12) (Study ID ● 471).

Peters et al. (1997) observed a weak, but statistically significant decrease in evening peak expiratory flow (-1.67 range: -2.76 to -0.58) for a 25 ppb incremental increase in 5-day mean levels (mean 27.2 ppb; max 146.2 ppb) of SO_2 in the Czech Republic. Only one monitor was used to measure SO_2 concentrations in this city-wide study. The criteria used to check the validity of PEF measurements was substantially more lenient than other studies (Study ID ● 472).

Other asthma incidence

Using neural networks, Moseholm et al. (1993) investigated function changes in asthmatics and low-level SO_2 and other atmospheric factors. The range of the 24hr means of ambient SO_2 was 6.5 to 6.8 ppb. Over the 8-month period, subjects kept a diary detailing symptoms, lung function, medicine intake and tobacco smoking. Lung function was associated with ambient SO_2 concentrations of SO_2, as well as with temperature, relative humidity, and medicine intake. Increased SO_2 concentrations corresponded to decreased peak flow levels at concentrations above 15 ppb (Study ID ◆ 333).

Buchdahl et al. (1996) reported significant associations (OR 1.12 95%CI 1.06-1.17) between variations in daily SO_2 concentrations (5.3 ppb incremental increase; mean 8.4±5.3 ppb) and the incidence of acute wheezy episodes, after adjustment for season. However, other compounds in air pollution were not considered, and the reliability of the outcome diagnoses was questionable (Study ID ● 364).

Tarlo et al. (2001 assessed possible associations between ambient SO_2 concentrations and symptomatic colds in the production of asthma exacerbations. An association was observed from March to November (no association reported, p<0.1) between higher levels of SO_2 (4.94 ppb vs. 3.04 ppb) for 3 days before asthma exacerbation with a cold compared with asthma exacerbations without a cold. Cold diagnosis depended on diary reporting by subjects and there are limitations in exposure assessment (Study ID ●433).

Neukirch et al. (1998) reported significant associations between an incremental 19 ppb increase (mean

8.3±5.2 ppb; range 1.7-32.0 ppb) in SO_2 (5 day lag) and incidence of wheeze (OR 1.35 95%CI 1.10-1.81) and nocturnal cough (OR 1.34 95%CI 1.00-1.79) (lag days 3 and 5) in asthmatics in Paris in the winter. SO_2 levels also correlated significantly with decreased morning peak expiratory flow. Sample size was small: n=40, who were then divided into two groups. Only significant findings were reported. Selection bias may be a factor. Little detail is given on the SO_2 data and monitoring (Study ID ● 455).

Other hospital admissions
Ponka (1991) found significant correlations of daily concentrations of SO_2 (daily mean 7.3±4.8 ppb; range 0.08-36.1 ppb) and admissions to emergency wards in the elderly (7% more admissions during higher pollution; confidence interval not reported). However, cold weather and other pollutants also had effects and SO_2 was highly correlated with these factors (Study ID ● 453).

No associations observed
Kesten et al. (1995) investigated a relationship between emergency room visits for asthma and atmospheric pollutant concentrations. SO_2 concentrations over the yearlong study were measured to be between 0 and 1.5 ppm (0-1500ppb). No association was observed between SO_2 and emergency room visits; however, some association was observed with other air pollutants (Study ID ◆ 023).
Moolgavkar et al. (1997) investigated air pollution and hospital admissions for chronic obstructive pulmonary disease (COPD) in Minneapolis-St. Paul, Minnesota and Birmingham, Alabama from 1986 to 1991. SO_2 analysis was not done in Birmingham due to a large amount of missing information. There were no significant associations between hospital admissions (increase in admissions 1.6% 95%CI -0.1%-3.3%) and 3.5 ppb incremental increase in SO_2 concentrations (mean 4.8-6.6 ppb). The study authors point out that confounding by other pollutants cannot be ruled out. Very little detail is given regarding the exposure monitoring (Study ID ●331).
Schouten et al. (1996) investigated the short-term relationship between air pollution and the daily number of emergency hospital admissions for respiratory disease in Amsterdam and Rotterdam as part of the APHEA project. Results were inconsistent with both negative and positive associations observed between SO_2 concentrations and hospital admissions for respiratory disease. Only a few of the negative associations reached significance. The authors suggest this may be due to the low levels of SO_2 (24 hour mean Amsterdam: 11 ppb; Rotterdam: 15 ppb) and low admission counts during the study (Study ID ●353).
Tenias et al. (1998) found no significant associations between incremental 10 ppb increases in SO_2 (24-hour mean 10.2 ppb) and emergency room visits for asthma (all confidence intervals included 1.0) in an ecological study in Valencia, Spain as part of the APHEA project. Limitations of the study include exposure assessment and a small number of asthma visits (Study ID ● 425).
Burnett et al. (1999) reported no statistically significant associations between daily hospital admissions for respiratory, cardiac, cerebral vascular, and peripheral vascular diseases and daily 10 ppb incremental increases in SO_2 (mean 5.4 ppb). Small percentage increases in hospital admissions attributed to SO_2 could be almost

entirely explained by other atmospheric variables. Limited detail is reported on the exposure monitoring and confidence intervals are not reported (Study ID ● 454).

Harre et al. (1997) reported no significant association between incremental 1.7 ppb increases in ambient SO_2 levels (baseline exposure 0-15 ppb) in Christchurch, New Zealand and either morning or evening peak expiratory flow rate. Selection bias is a possibility in this study and there is some inconsistency regarding the number of subjects recruited from various methods. Training effects were not considered and exposure assessment was taken from the results of one monitor (Study ID ● 470).

Prescott et al. (1998) reported no statistically significant change in the risk of hospital admissions associated with a 10 ppb incremental increase in SO_2 as a moving average of the previous 3 days in Edinburgh (daily mean 14.5±9.0 ppb; 8.3±5.6) ppb. There is some question regarding the validity of the SO_2 data. This and the limited number of monitoring sites suggest that exposure assessment is a major limitation of this study (Study ID ●473).

Hernandez-Garduno et al. (1997) reported that ambient SO_2 levels were negatively correlated with patient visits to clinics in Mexico City. No further analysis was done on SO_2. Associations and SO_2 concentrations were not reported (Study ID ● 475).

Holmen et al. (1996) reported no statistically significant correlations between emergency department visits for asthma and daily ambient SO_2 levels (mean 1.3 ppb summer; mean 2.6 ppb winter; 24-hour range: 0.1-30 ppb). SO_2 monitoring was well described, but measurements were made 10 m off the ground and not the inbreathing zone of patients. Exposure assessment is a limitation of this study, and outcome classification may be questionable. Confidence intervals were not reported (Study ID ●478).

Sheppard et al. (1999) reported no significant associations between an incremental 10 ppb increase in ambient SO_2 levels (daily mean: 14.5±9.0 ppb; 8.3±5.6 ppb) in Seattle and hospital admissions for asthma. However, there was an association between the control admissions (appendicitis) and SO_2 levels. Statistical significance of results was not reported. Exposure assessment was a limitation in this study and the study authors speculate that local emissions in the vicinity of the monitor influenced SO_2 measurements (Study ID ● 482).

Castellague et al. (1995) reported no statistically significant associations between incremental 9.5 ppb increases in ambient SO_2 levels (daily means summer 40.8; winter 52.0 ppb) and emergency room visits in Barcelona, Spain. There were some issues regarding the diagnosis of asthma and chronic obstructive pulmonary disease, which may have lead to outcome misclassification. Limited detail was reported on SO_2 monitoring and measurement (Study ID ●484).

Industrial

Donoghue and Thomas (1999) examined the effect on hospital presentations for asthma of brief spikes in ambient SO_2 concentrations near copper and lead smelters. No relationship was observed between peak ambient SO_2 concentrations up to 3300 ppb and hospital presentations or admissions for asthma (Study ID ◆ 007).

Zuskin et al. (2000) examined differences in respiratory symptoms in mail carriers in Croatia exposed to an average of six hours of ambient air pollution with concentrations up to 190 ppb compared with control subjects who were not. They observed an increase in the prevalence of upper airway symptoms and nasal catarrh. Measured ventilatory capacity tests were also lower than expected, particularly FEF_{25}, FEF_{50}, and FVC, in both smokers and non-smokers (Study ID ◆ 009).

A study of nickel smelter workers investigated the prevalence of adverse pulmonary function parameters compared to controls (Holness et al., 1985). An average of 0.47 ppm SO_2 was measured in the nickel smelter. Higher prevalence of cough, dyspnea, lower baseline function, as well as decreases in FVC and FEV_1 over the workweek were observed in the smelter workers compared to controls. Baseline airflow rates and FVC and FEV_1 varied over the workweek with levels of SO_2 and particulate matter. During a smelter shutdown, a slight increase in lung function was observed in both smelter workers and controls (Study ID ◆ 016).

Lung function was measured in temporary workers in greenhouses with SO_2 concentrations ranging between 0.15 and 0.66 ppm (Likas et al., 2001). No significant effects on lung function were observed with temporary work in the greenhouse (Study ID ● 017).

Lawther et al. (1974 a, b, c) did a series of pulmonary measurements every working day for five years on four normal subjects employed in central London. Measurements included FEV_1, MMF, and peak expiratory flow. Day-to-day changes in pulmonary measurements were compared with ambient levels of smoke and SO_2. MMF showed the most consistent association with pollution levels. Respiratory infections had a substantial effect on pulmonary measurements. Outdoor exercise had some association with decreases in FEV_1 and MMF. However, associations between pulmonary measures and pollution levels were generally not consistent, even for seasonal variations. None of the associations were reported to be significant (Study ID ● 029, 30, 31).

Case-reports

Several case-reports outline the effects of sudden, accidental exposure to high levels of SO_2.

Harkonen et al. (1983) report the experiences of seven men accidentally exposed to high concentrations of SO_2 in a pyrite mine explosion. Exposure concentrations are unknown and duration of exposure was estimated at 20 to 25 minutes. Nine men were initially exposed. Two subsequently died. Immediately following exposure, thoracic pain and coughing were observed. One week after exposure, the greatest decreases in FVC, FEV_1 and MMFR were observed. No further decrement in lung function was observed by three months after the exposure. Four years after the exposure, the lung function of the workers increased, but never up to pre-accident levels. Four of seven workers showed signs of bronchial hyperreactivity (Study ID ● 021). Piirila et al. (1996) report a subsequent follow-up with the same subjects at 13 years post-exposure. Of the six surviving workers, one had normal spirometry, two had obstruction, and three had obstructive and restrictive ventilatory impairment. A histamine challenge test was performed. One worker could not perform the test due to bronchial constriction. The remaining five workers showed bronchial hyperreactivity (Study ID ◆ 001).

Rabinovitch et al. (1989) report the cases of two miners exposed to high concentrations of SO_2 in a mine explosion. Three weeks after the incident, both miners showed evidence of severe airway obstruction, hypoxemia, reduced exercise tolerance, and evidence of active inflammation. Over the next 12 months, some improvement in lung function was observed. However, no subsequent improvement was observed three years after the exposure incident (Study ID ◆ 272).

Woodford et al. (1979) present the case of a previously healthy, non-smoking young man exposed to a short, but high concentration of SO_2 (concentrations unknown). Immediately during and after exposure, rhinorrhea, cough, and pulmonary edema were observed with subsequent development of severe, irreversible pulmonary obstructive syndrome. The man was treated with oxygen and released with a clear chest roentgenogram. After a few days, progressively worse dyspnea and severe cough were noted. Upon re-hospitalization, hyperinflation, coarse basilar rales, purulent sputum, and dyspnea at rest were noted. The patient was monitored over a three-year period and the symptoms appeared to stabilize. However, he was diagnosed with persistent obstructive airways disease including forced expiratory wheezing (Study ID ● 269). Charan et al. (1979) observed five subjects who sustained exposure to high concentrations of SO_2 (actual concentrations unknown) in an industrial accident. Two of the five died immediately. The three survivors were subjected to lower concentrations and underwent pulmonary function testing at regular intervals after the incident. Acute symptoms of the three survivors included irritation and soreness of the throat, tightness in the chest, and intense dyspnea, as well as decreased breath sounds, and diffuse rales. Subsequent tests indicated severe irreversible airway obstruction in one subject and mild airway obstruction in another subject. The fifth subject, a firefighter who had been wearing respiratory apparatus at the time of exposure, showed no acute or chronic pulmonary function abnormalities (Study ID ● 270).

Galea (1964) reported the case of a 35-year old man who died 17 days after accidental exposure to high levels of SO_2. Several days after exposure, the man was discharged from hospital in satisfactory condition. 10 days after discharge, the man was re-admitted with dry, irritable, tiresome cough, dyspnea, copious amounts of mucus, rales on both lung bases, and audible wheezing. The man died in hospital. An autopsy revealed a uniformly marble appearance and a feathery, pillowy consistency of the lungs. In addition, there was extensive tracheobronchitis, airway lesions, intense submucosal gland activity, and extension of alveoli and air sacs (Study ID ● 271).

Respiratory System – Biochemical

Most of the studies investigating the effects of SO_2 on pulmonary biochemistry did so in an effort to increase understanding of the mechanism of action. The clinical significance of reported biochemical effects is often uncertain and not discussed in the studies.

Clinical studies

Speizer and Frank (1966a) measured the absorption and desorption of SO_2 in the upper respiratory tracts of healthy subjects breathing by nose. They observed that SO_2 concentration in the respiratory tract decreased upon inspiration (from 16.1 ppm in the breathing mask to 13.8 ppm inside the nose to undetectable in the oropharynx). Expired gas in the pharynx was virtually free of SO_2, but expired gas exiting the nose had picked up 2.0 ppm SO_2. They concluded that SO_2 is removed by the nose during inhalation and some is desorbed on exhalation (Study ID ◆ 033).

Field et al. (1996) investigated the mechanism of SO_2-induced bronchoconstriction in asthmatic subjects by exposing them to 0.5 to 8.0 ppm SO_2 with and without previous exposure to an opioid or a cyclooxygenase inhibitor. Response was measured as the dose of SO_2 required to induce a 35% fall in specific airways conductance. SO_2 responsiveness decreased with the opioid and increased with the cyclooxygenase inhibitor (Study ID ◆ 052).

Bechtold et al. (1993) exposed adult asthmatics to either 1 ppm or 7 ppm SO_2 for 10 to 20 minutes every other day for three weeks to investigate the S-sulfonate levels in nasal lavage fluid as a potential biomarker of SO_2 exposure. They found that S-sulfonate levels were statistically significantly elevated relative to control group levels. However, the levels did not accumulate over time, suggesting continuous clearance from the nasal passages. The levels of S-sulfonate observed in the nasal lavage fluid are approximately three orders of magnitude higher than levels measured in plasma after exposure to similar concentrations of SO_2, suggesting that S-sulfonate in nasal lavage fluid could be a good short-term biomarker for SO_2 exposure (Study ID ◆ 066).

Sandstrom et al. (1989a) used bronchoalveolar lavage (BAL) fluid to investigate the effect of SO_2 on the healthy human lung. 24 hours after exposure to 4 ppm SO_2 for 20 minutes, increased alveolar activity was observed in BAL fluid. Even greater alveolar activity as well as increased numbers of macrophages and lymphocytes were observed 24-hours after exposure to 8 ppm for 20 minutes. After both exposures, all cell activity returned to normal within 72 hours (Study ID ◆ 083). Further study found significant increases in lysozyme-positive macrophages, lymphocytes, and mast cells in BAL after exposure to 8 ppm SO_2 for 20 minutes during exercise (Sandstrom et al., 1989b). Peak values were observed 24 hours after exposure and cell numbers returned to pre-exposure values within 72 hours (Study ID ◆ 090). A dose-dependent increase in the number of mast cells, lymphocytes, lysozyme-positive macrophages and total number of macrophages was observed in BAL fluid after exposures to 4, 5, and 8 ppm for 20 minutes, but not up to 11 ppm (Sandstrom et al., 1989c) (Study ID ● 091).

Witek and Schachter (1985) investigated the mechanism of SO_2 bronchoconstriction by exposing mildly asthmatic subjects to 1 ppm SO_2 for 40 minutes and to methacholine. They found a significant correlation between the dose of SO_2 and the dose of methacholine required to reduce flow rates to 60% of vital capacity. The authors postulate that there is a relationship between the mechanism of response to SO_2 and to methacholine (Study ID ◆ 085).

Lazarus et al. (1997) postulate that cysteinyl leukotrienes contribute to the bronchoconstriction seen in asthmatics during SO_2 exposure. They found that the leukotriene receptor antagonist zafirlukast inhibited SO_2-induced bronchoconstriction in 12 subjects exposed to up to 8.0 ppm SO_2 for 4 minutes, supporting their postulation (Study ID ◆ 321).

Non-clinical studies

Guinea pigs

Riedel et al. (1988) exposed guinea pigs to between 0.1 and 16.6 ppm SO_2 for 8 hours a day for 5 consecutive days to investigate the effect of SO_2 exposure on bronchial sensitivity to inhaled antigen. 67% to 100% of the SO_2-exposed animals exhibited positive bronchial reactions to inhaled antigen compared to only 7% of the control animals. In addition, the extent of bronchial obstruction as well as concentrations of antigen-specific antibodies in serum and bronchoalveolar fluid were greater in animals exposed to SO_2 at all levels compared to controls (Study ID ◆ 133).

Haider (1985) investigated the lipid metabolism in the organs of guinea pigs exposed to 1 hour of 10 ppm a day for 30 days. Increased concentrations of cholesterol, total lipids and gangliosides and a decrease in phospholipids were observed (Study ID ◆ 163).

Atzori et al. (1991) investigated the mechanism of SO_2-induced bronchoconstriction using guinea pig lungs. Guinea pigs were exposed to 250 ppm SO_2 for 10 minutes after treatment by a cyclooxidase inhibitor or an H1-receptor agonist. Neither compounds attenuated the SO_2-induced bronchoconstriction, suggesting that the bronchoconstriction is a result of a local effect on sensory nerves, possibly dependent on the release of sensory neuropeptides (Study ID ◆ 178).

Halinen et al. (2000) examined the effects of exposing guinea pigs to ten minutes each of 1, 2.5, or 5 ppm SO_2 in cold air. In the group exposed to cold air with SO_2 there was a significantly lower proportion of macrophages in BAL white cells in comparison to the group exposed to cold air without SO_2 (Study ID ◆ 245).

Hajj et al. (1996) exposed Dunkin Hartley guinea pigs to 500, 1000, 1500, and 2000 ppm SO_2 to investigate the role of tachykinins in the bronchoconstriction response. They concluded that tachykinin release from sensory endings does play a role in SO_2-induced bronchoconstriction in anaesthetized guinea pigs. Exposure duration was not clearly stated. There is limited information on the experimental conditions and design (Study ID ●370).

Ito et al. (1995) exposed Hartley guinea pigs to 800 ppm for 2 hours and observed that direct epithelial injury from SO_2 inhalation results in loss of epithelial cells and an increase in permeability. In addition, they suggest that inflammatory cells may promote rather initiate bronchial responsiveness. The information provided in the paper suggests that Good Laboratory Practice guidelines were not followed (Study ID ● 452).

Rats

Kahana and Aronovitch (1968) investigated the effects of SO_2 exposure on pulmonary surface tension. After exposure to 800 ppm SO_2 for 3 hours, a small but significant reduction in maximal and minimal surface tension but no respiratory distress or pathological changes in the lungs were observed. After exposure to 1225 ppm for 2 hours, pulmonary edema was observed. In addition, two of the rats exhibited severe and persistent dyspnea while the others had transitory and less severe respiratory difficulty. There was a greater drop in surface tension at the higher exposure (Study ID ◆ 155).

Vai et al. (1980) investigated the effects of exposure to 600 ppm SO_2 for 30 to 100 hours on rat tracheobronchial epithelium. They observed a considerable increase in mucosal permeability in the tracheal epithelium and epithelium of the main bronchi both in vivo and in vitro. This increase was reduced but still present three months post-exposure (Study ID ◆ 206).

Langley-Evans et al. (1996) investigated the potential of SO_2 to be a glutathione (GSH) depleting agent. Rats were exposed to SO_2 concentrations between 5 and 100 ppm for 5 hours a day for 7 to 28 days. The lowest concentrations (5ppm) did not result in lung injury or inflammation, but levels of GSH were lower in the lung, liver, heart and kidney. In addition γ-glutamylcysteine, glutathione peroxidase, glutathione S-transferase, and glutathione reductase activities were all reduced. At 100 ppm there was evidence of lung inflammation and GSH levels were lowered as well as enzyme activity. However, at 50 ppm, GSH levels were the same relative to controls, although enzyme activity was lowered (Study ID ◆ 251).

Husain and Dehnen (1978) examined the effect of SO_2 on benzo(a)pyrene metabolism in the lungs of rats exposed to 46.5 ppm continuously for up to 4 weeks. Benzo(a)pyrene metabolism was measured as the activity of aryl hydrocarbon hydroxylase (AHH). No change was observed in AHH activity between the exposed and control animals (Study ID ◆ 252).

The effect of SO_2 exposure on four rat enzyme systems was investigated by Barry and Mawdesley-Thomas (1970). After exposure to 300 ppm SO_2 for six hours a day for 10 days, the acid phosphatase activity in the free alveolar cells of the lung parenchyma was markedly increased. There was also a slight increase in the activity of β-glucuronidase, β-galactosidase, and N-acetyl-β-glucosaminidase in some free alveolar cells in the peribronchiolar region (Study ID ● 181).

Gause and Barker (1978) exposed rats to SO_2 concentrations from 5 to 20 ppm continuously for a week to investigate the uptake of SO_2 in the nasal mucus and the subsequent effects on the electrophoretic properties of nasal mucus glycoproteins. In the first 30 minutes of exposure, 90% of the inhaled SO_2 remained in the nasal mucus with 10% found in the plasma or serum. The ratio of SO_2 concentrations in the mucus to those in the serum leveled off after 1 to 4 hours of exposure to approximately 3:1. Electrophoretic gels of nasal mucus were collected immediately following exposure as well as at 8 days post-exposure. A dose-related increase in new bands on the electrophoretic gels were observed at both times, suggesting polymerization of mucus glycoproteins (Study ID ● 193).

To investigate the effects of SO_2 exposure on the surface properties of lungs, rats were exposed to 627-751 ppm SO_2 for either a single 3-hour exposure or 9 exposures of 4 hours each at 257-450 ppm (Kahana and Aronovitch, 1966). A reduction of surface forces and a decrease in mean transpulmonary pressures was observed in the single exposure animals. Changes in surface properties were unclear in the multiple exposure group due to differences in characteristics between the exposure and control groups (Study ID ● 262).

Mice

In an investigation of the effect of SO_2 on the pathogenesis of upper respiratory viral infection, mice were exposed to 0.03 to 0.1 ppm SO_2 for four weeks (Ukai, 1977). The inflammatory response to the virus was more rapid and severe in SO_2-exposed mice than in those not exposed to SO_2. In addition, regeneration and antibody appearance were initiated sooner and the HI titer developed more rapidly and reached higher levels in the exposed mice than the non-exposed mice. Animals that were exposed to SO_2 but not infected with the virus exhibited a sixfold increase in the number of goblet cells in the nasal epithelial cells (Study ID ◆ 207).

Hamsters

Skornik and Brain (1990) investigated the effect of exercise and 50 ppm SO_2 exposure on pulmonary macrophage endocytosis in Syrian golden hamsters. A significant reduction was observed only after 40 minutes of continuous running while breathing 50 ppm SO_2. There were no changes with non-exercise exposure to SO_2 or between exercise and no exercise without SO_2 exposure (Study ID ▲ 374).

Squirrels

Biochemical changes were investigated in the trachea, lungs and heart of squirrels exposed to 500 ppm SO_2 for 5 minutes (Rana et al., 1979). Changes in lung lipids caused changes in the surface tension of the lungs. In addition, membrane permeability was altered, resulting in changes in protein content (Study ID ● 147).

Chickens

Majima et al. (1985) investigated the elastic recoil distance both *in vivo* and *in vitro* of nasal mucous from chickens exposed to 6 ppm SO_2 for 16 hours a day for 7 days. They observed a decrease in the recoil distance *in vivo*, but not *in vitro* after SO_2 exposure (Study ID ● 149).

Okuyama et al. (1979) exposed chickens to levels of SO_2 ranging from 3.4 to 18.5 ppm for between 1 and 14 days to investigate any histological changes in the tracheal mucosa. At all exposure levels, there was an increase in infiltrating mononuclear and polymorphonuclear cells, acid phosphatase positive cells, the number of plasma cells and lymphocytes, acid mucins, and in the number of mitotic figures. There were also changes in mucus type and a decrease in neutral mucins. At the highest concentration (18.5 ppm), the mucosa-to-wall ratio doubled and some mucosal damage was observed (Study ID ● 199).

Bauer (1981) exposed chickens to 350 to 400 ppm SO_2 for three hours to investigate the biochemistry of tracheobronchial secretions. A significantly increased mucus output (320%) was observed in the chickens exposed to SO_2 compared to controls; however, glycoprotein output increased by only 50%. Therefore, the glycoprotein concentration was lowered to approximately one third of the concentration in the control group. No differences were observed in the carbohydrate pattern of the glycoproteins between the exposed and non-exposed chickens (Study ID ● 221).

Dogs

Man et al. (1986) investigated the effect of SO_2 exposure on the bioelectric and barrier properties of the tracheal epithelium. After exposure to 100 ppm SO_2 for 75 minutes, there were few bioelectric changes in the trachea of dogs. However, after exposure to 500 ppm for the same amount of time, adverse changes to the bioelectric properties were observed, as well as nonelectrolyte permeability (Study ID ◆ 150).

No effects observed

Azoulay et al. (1980) examined the effects of continuous exposure for 1 to 49 days to 2 ppm SO_2 alone and in mixtures with 2 ppm NO and NO_2 on lung structure and blood-oxygen affinity in rats. They observed no difference in blood erythrocyte variables and oxyhemoglobin dissociation curves between exposed animals and controls for any gases or mixtures of gases. No changes in lung structures were observed (Study ID ◆ 225).

Epidemiology

No epidemiology studies reported biochemical effects.

Respiratory System – Structural

Clinical studies

Kienast et al. (1994a) investigated the effect of SO_2 at different concentrations on ciliary beat frequency. Cells taken from volunteers' noses were exposed for 30 min to 2.5 to 12.5 ppm at 37°C and 100% humidity. A dose-dependent decrease in ciliary beat frequency was observed from low to high concentrations of SO_2 (Study ID ◆ 320).

In a similar study, Kienast et al. (1996) exposed cells taken from the noses of 12 healthy volunteers to 0, 2.5, 5.0, 7.5, 10.0, and 12.5 ppm for 30 minutes. A dose-dependent decrease in ciliary beat frequency was observed, suggested to be a result of the increase in hydrogen ion concentration in the culture medium (Study ID ● 427).

Riechelmann, et al. (1994) observed a concentration–dependent reduction in ciliary beat frequency in human nasal cells following exposure to 2.5, 5, 7.5, 10, and 12.5 ppm SO_2 for 30 and 120 minutes (Study ID ● 466).

Carson et al. (1985) examined the appearance of compound cilia in the nasal mucosa in normal human subjects following exposure to 0.75 ppm for 2 hours. They observed increases in the prevalence of compound cilia and a statistically significant association between SO_2 exposure and compounding of nasal epithelial cilia. They postulate that compound cilia are an acquired defect that could be used as a possible biomarker of SO_2 exposure (Study ID ● 046).

Non-clinical studies

Guinea pigs

Riechelmann et al. (1995) looked at correlations between functional alterations and morphological changes of the mucociliary system following SO_2 exposure. Guinea pigs were exposed to concentrations of 3, 6, 9, and 14 ppm for 30 minutes. There was a dose-dependent decrease in mucociliary activity in the exposed animals. However, only minor morphological changes were observed at the lowest exposure level. At higher exposure levels, epithelial sloughing, intracellular edema and mitochondrial swelling, ciliary cytoplasmic extrusions and a widened intercellular space were observed (Study ID ● 132).

Rats

Knauss et al. (1976) exposed rats to 600 and 700 ppm SO_2 for 3 hours a day equaling 9, 18, or 30 hours of cumulative exposure. A significant increase in the amount of solid material recovered by bronchial lavage was observed with increasing exposure time (Study ID ◆ 250).

Stratmann et al. (1991) exposed Wistar rats to 800 ppm SO_2 for 8 hours and observed the effects on the tracheal epithelium via electron microscopy. They observed a gradient of decreasing damage in the tracheobronchial tree in the peripheral direction. The most severe lesions (detached and necrotic cells and missing cilia and goblet cells) were observed in the trachea epithelium. The clinical significance of these results is unclear (Study ID ◆ 305).

Gross et al. (1969) exposed rats to very high concentrations of SO_2 (2500 and 4000 ppm) for 15 minutes to determine any morphologic evidence of alveolar edema. Evidence of edema was found in the separation of the surface epithelium from the alveolar septum, suggesting that development of edema was very rapid (Study ID ● 166).

Pariente (1980) studied the permeability of rat tracheobronchial epithelia both *in vivo* and *in vitro*. Rats were exposed to 600 ppm SO_2 for 100 hours or 1000 ppm for 4 hours. The four hours exposure to 1000 ppm was not lethal, but induced acute bronchitis and bleeding of the rhinopharynx. Chronic tracheobronchial injuries were observed after 600 ppm for 100 hours. After three months in a clean-air atmosphere, recovery was complete. However, there was increased permeability of the tracheobronchial epithelium both *in vivo* and *in vitro* (Study ID ● 210).

Hong (1996) observed no significant changes in cell count, LDH, total protein, CC16, and lysozyme in bronchoalveolar lavage fluid in rats exposed to 30 or 50 ppm for 4 or 12 hours. Details of the experimental methods were lacking (Study ID ● 447).

Farone et al. (1995) reported substantially increased numbers of polymorphonuclear leukocytes in rat tracheas after 1 day of exposure to 230 ppm SO_2. The study attempted to discover a mechanism for SO_2-induced chronic bronchitis. The study did not follow Good Laboratory Practice guidelines (Study ID ● 477).

Mice

Giddens and Fairchild (1972) examined mice from a defined flora colony with no disease (DF) and conventional mice with mild upper respiratory tract disease (CO) after exposure to 10 ppm SO_2 for 4 to 72 hours. After 24 hours of exposure, the olfactory mucosa was reduced to half the thickness seen in the control animals. In addition, olfactory hairs were smaller in number and height than controls. The CO mice at 24 hours exhibited complete necrosis of the olfactory mucosa. After 48 to 72 hours, DF mice exhibited severe rhinitis accompanied by serous exudation in the nasal cavity and desquamation of respiratory epithelial cells. The CO mice exhibited greater necrosis of the nasal mucosa at 48 to 72 hours than at 24 hours (Study ID ◆ 191).

Weiss and Weiss (1976) found a statistically significant increase in static lung compliance in mice after exposure to 40 ppm SO_2 for 6 to 9 days. Other effects were not statistically significant (Study ID ● 208).

Min et al. (1994) observed increasing injury to the olfactory epithelium in ICR mice as the duration of exposure to 20 ppm SO_2 increased from 30 to 60 to 120 minutes. Injuries included edema, loss of cilia, epithelial thinning, and epithelial desquamation in mice exposed for 60 and 120 minutes. No changes were observed in mice exposed for 30 minutes. Little detail is given on the experimental methods of treatment of animals as per Good Laboratory practice guidelines (Study ID ● 287).

Hamsters

Asmundsson et al. (1973) investigated the morphologic changes produced by repeated injury to airway epithelial cells by SO_2 and the time course of those changes. Hamsters were exposed to concentrations of 40, 100, 200, 250, and 400 ppm for 5 hours a day, five days a week for six weeks. Animals were examined frequently to assess time of any changes seen. Epithelial damage was observed in large airways at all levels of exposure after 1 week. A sequence of changes from loss of cilia at 1 to 2 days of exposure to squamous metaplasia after 2 to 4 weeks was observed at concentrations above 100 ppm (Study ID ● 198).

Rabbits

Blanquart et al. (1995) observed significant ultrastructural alterations of ciliated cells after a 1-hour exposure to 10 and 30 ppm SO_2 in Fauve de Bourgogne rabbits. Ciliary activity was significantly inhibited. These findings suggest a mechanism of action of SO_2 toxicity (Study ID ◆ 463).

Dalhamn and Strandberg (1961) reported no effect on ciliary movement in rabbit trachea after inhalation of 200 ppm SO_2 for 45 minutes. However, ciliary movement stopped when 10 ppm SO_2 or greater was blown directly onto the trachea. Information on the experimental protocol was lacking (Study ID ● 294).

Strandberg (1964) reported differences in the absorption of high concentrations of SO_2 and low concentrations in the respiratory tract in rabbits. Concentrations ranged from 0.05 to 700 ppm; however, there is very little detail given regarding the dosing regime or exposure duration. Other information on the experimental protocol is also lacking (Study ID ● 417).

Dogs

Frank et al. (1967) investigated the uptake of $^{35}SO_2$ into various body fluids in dogs exposed to 22±2 ppm for 30 to 60 minutes. There was limited detail on the experimental protocol and Good Laboratory Practice guidelines were not followed (Study ID ● 286).

Balchum et al. (1959) reported that dogs breathing $^{35}SO_2$ through the nose and mouth retained a smaller proportion of the inhaled gas in the trachea, lungs, hilar lymph nodes and liver and spleen than dogs breathing similar concentrations via tracheostomy. However, only one dog was exposed to each exposure level (1.1 to 141 ppm) and duration (20 to 40 minutes) and details on all areas of the experimental protocol are lacking (Study ID ● 418).

Human in vitro studies

Knorst et al. (1996a) reported functional impairment of human alveolar macrophages after exposure to 1.0, 2.5, and 5.0 ppm SO_2 for 30 minutes. The clinical and toxicological significance of the results was not clear (Study ID ◆ 308).

Knorst et al. (1996b) observed changes in alveolar macrophage and blood monocytes chemotactic activity upon exposure of human macrophages from subjects with bronchial carcinoma to 0.5, 1.5, and 2.5 ppm SO_2 for 15 minutes. Cell viability was not affected. The clinical significance of these results is unclear, as it cannot be assumed that in vivo studies would produce the same results (Study ID ● 319).

Epidemiology

No epidemiology studies were found that reported solely structural effects of SO_2 exposure. Any structural effects were reported as the cause of functional effects found only after autopsy. These results were considered in the section ***Respiratory system-Functional.***

Respiratory System – Signs and Symptoms

Several studies focussing on other respiratory effects also reported signs and symptoms of respiratory distress.

Clinical studies

Effects observed

Adult human subjects were exposed to SO_2 concentrations between 0.5 and 5 ppm for 1 to 5 minutes both with and without exercise (Kreisman et al., 1976). Breathing was through the mouth. After a 3-minute exposure to 3 ppm or 5 ppm either at rest or during exercise, 15 of 18 subjects reported dryness, irritation or burning of the throat. Frequently, subjects would report an urge to cough immediately after SO_2 inhalation. Symptoms were also reported when breathing air without SO_2, but not when breathing 0.5 ppm or 1.0 ppm SO_2 (Study ID ◆ 039).

Andersen et al. (1974) investigated the human response to controlled levels of SO_2. They exposed adults to between 1 and 25 ppm SO_2 for 6 hours a day for three consecutive days. Discomfort was reported, proportional to SO_2 concentration (Study ID ◆ 063).

Balmes et al. (1987) investigated the relationship between bronchoconstriction in asthmatic adults and the duration and concentration of SO_2 exposure. Subjects were exposed to 0.5 and 1.0 ppm for 1, 3, and 5 minutes. After exposure to 1.0 ppm for 1 minute, a quarter of the subjects developed chest tightness. After exposure to 0.5 ppm for 3 and 5 minutes or exposure to 1.0 ppm for 3 minutes, seven of eight subjects showed wheezing, chest tightness and dyspnea, and used an inhaled bronchodilator (Study ID ◆ 064).

Witek et al. (1985) investigated subjective responses to concentrations of SO_2 less than 1 ppm for 40 minutes in healthy and asthmatic subjects. Asthmatics reported chest tightness, wheezing, dyspnea and cough, while the healthy subjects reported taste and odour complaints. There was an increase in the number and severity of the complaints associated with increasing concentration (Study ID ◆ 093).

No effects observed

Kagawa (1983) observed no symptoms in healthy adults after exposure to 0.15 ppm SO_2 for 2 hours with intermittent light exercise (Study ID ◆ 072).

Non-clinical studies

Amdur (1954) observed few signs of respiratory distress in guinea pigs exposed to 89 ppm SO_2 for 8 to 16 hours (Study ID ● 125).

Matsumura (1970) exposed guinea pigs to 20, 60, 180, and 330 ppm SO_2 for 30 minutes. Signs of irritation, such as sneezing, rubbing eyes or noses, or uneasiness in the animals were observed only after exposure to 330 ppm. Signs of irritation disappeared within a few minutes of exposure. There were no significant signs of respiratory distress (Study ID ● 142).

Matsumura et al. (1972) examined the effects of pre-exposure to SO_2 and other air pollutants on the sensitivity of guinea pigs to inhaled acetylcholine. Guinea pigs were exposed to 450, 600, and 700 ppm for 30 minutes. No differences in respiratory signs were observed between the control group and the groups exposed to SO_2 at any concentration (Study ID ● 144).

Epidemiology studies

Effects observed

Cohen et al. (1974) carried out a telephone survey to investigate the reporting of irritative symptoms during well-publicized and unpublicized periods of moderately elevated air pollution (peak hourly SO_2 = 0.11 to 0.15 ppm) and a period of low pollution (peak hourly SO_2 = 0.02 to 0.04 ppm). Significant increases in eye irritation, throat irritation, chest discomfort, shortness of breath, restricted activity, and medical visits were observed during both elevated pollution episodes compared to the control (low pollution) period. ORs and confidence intervals were not reported (Study ID ◆ 011).

Carnow et al. (1969) investigated a potential dose-response relationship between levels of pollution as measured by SO_2 concentrations and respiratory morbidity in patients with chronic bronchopulmonary disease. SO_2 concentrations were measured at up to 0.30 ppm over the 10 month study period. They observed a dose-response association between SO_2 concentrations and percent person-days of illness; the rate of illness was 50% greater on days when SO_2 concentrations were 0.25 ppm than on days when SO_2 concentrations were 0.04 ppm or lower. Associations and confidence intervals were not reported (Study ID ◆ 010).

A study of nickel smelter workers investigated the prevalence of adverse pulmonary function parameters compared to controls (Holness et al., 1985). An average of 0.47 ppm SO_2 was measured in the nickel smelter. Higher prevalence of cough, dyspnea, and lower baseline function over the workweek were observed in the smelter workers compared to controls. Associations and confidence intervals not reported (Study ID ◆ 016).

No effects observed

Love et al. (1982) investigated the effect of increased exposure to SO_2 on respiratory illness in the Great Salt Lake basin, Utah. Annual mean ambient SO_2 concentrations were measured to be between 15 and 30.5 ppb. No relationship was observed between higher SO_2 concentrations and respiratory illness (Study ID ◆ 015).

Hoek and Brunekreef (1993) investigated the effect of winter air pollution episodes on respiratory effects of children. SO_2 concentrations were measured to be between 0 and 38 ppb. No association was observed the prevalence of acute respiratory symptoms and the concentrations of SO_2 or other compounds (Study ID ◆ 018).

Franklin et al. (1985) did an epidemiology study to investigate the health effects of acute exposure to transported air pollutants in asthmatic and non-asthmatic children. The study lasted 10 days and the levels of SO_2

were not reported. No statistically significant health effects were observed in relation to SO_2 (Study ID ● 004). Ayres et al. (1989) investigated the respiratory health effects of an acid transport event in January 1985 in the United Kingdom. SO_2 levels were measured to be between 19 and 40 ppb in a polluted area and 13 to 27 ppb in an unpolluted area. No increase in respiratory morbidity was observed between the two areas (Study ID ● 006). Complaints of shortness of breath, frequent colds, cough, sore throat, and chest tightness prompted a Health Hazard Evaluation of Portland cement plants to assess whether SO_2 exposure was the genesis of the problems (NIOSH, 1984). SO_2 levels at the plants were measured to be 1.03 ppm at one plant and between 0.2 and 1.8 ppm at another (Study ID ● 267).

Respiratory System – Other

Fairchild et al. (1972) investigated the effect of exposure to SO_2 on influenzal pneumonia in mice. Mice were exposed to concentrations ranging between 3.4 and 34.5 ppm continuously for up to 7 days. When SO_2 was administered after virus exposure, significantly increased incidence of pneumonia was observed at 19 ppm and greater. When the virus was administered after SO_2 exposure, a significant increase in pneumonia incidence occurred only at 25 ppm after 4 to 7 days of exposure. Histopathological effects were observed at SO_2 concentrations of 27 ppm and greater after 7 days of continuous exposure (Study ID ◆ 182). Suzuki (1969) exposed guinea pigs to 10 and 50 ppm SO_2 for 3 hours to compare the lung histamine and water content of exposed and non-exposed animals. No effect on water or histamine content of the lungs was observed at either exposure level (Study ID ● 126). Frank et al. (1969) investigated the uptake and desorption of $^{35}SO_2$ in the nose and mouth of dogs exposed to SO_2 concentrations between 1 and 50 ppm for 1.5 to 5 minutes. Virtually all the $^{35}SO_2$ was absorbed by the nose at all concentrations and times. Absorption by the mouth was concentration-dependent. Desorption was observed only from the mouth, with release of $^{35}SO_2$ gas observed up to 25 minutes post-exposure (Study ID ● 169).

Summary

The largest number of studies and consequently the most convincing evidence for adverse health effects of SO_2 exposure was found in effects on the respiratory system. Please refer to Tables 1 though 9 and Figures 1 to 7 for further summary.

Clinical

Of the 96 clinical studies investigating respiratory effects, 73 (76%) were ranked high or moderate.

Healthy subjects

There were no high quality studies looking at healthy humans in the time range of 1 to 10 minutes of exposure. There were, however, a number of moderate quality studies. Pulmonary effects in healthy humans starting at 0.75 ppm and up to 15 ppm were observed in clinical studies. These studies involved direct exposure to SO_2 with hyperventilation and/or exercise. There is some evidence that pulmonary effects are greater when exposure is through a mouthpiece (orally) than through the nose. Dryness, irritation and burning of the throat were observed at 3, *15, and 28 ppm in two moderate quality studies.*

Asthmatic subjects
Only one study of 27 investigating exposure for 1 to 10 minutes was rated high quality. This study noted a concentration-dependent change in respiratory function in asthmatics between 0.5 and 1 ppm with exposure during light to heavy exercise. The moderate quality studies also involved direct exposure, usually with exercise and/or hyperventilation. Small but significant pulmonary effects were observed in asthmatics at concentrations ranging between 0.1 ppm to 10 ppm. These effects were transitory and pulmonary function returned to normal after exposure ceased. Again, there is evidence that mouth breathing or oral exposure results in a great effect than nasal exposure.

Healthy subjects
Pulmonary effects were observed at concentrations as low as 1 ppm at exposures times between 11 and 30 minutes. Again, these effects were transitory. Three studies investigated the effects on cells from the respiratory system after exposure to concentrations between 2.5 and 8 ppm. Some effect was observed on these cells.

Asthmatic subjects
Again, only one study was ranked high for exposures between 11 and 30 minutes. Pulmonary function effects were observed in asthmatics upon exposure to 0.5 ppm SO_2 with moderate exercise.
Other studies suggest pulmonary effects with exercise at concentrations between 0.1 ppm and 1 ppm.

The weight of evidence for exposures up to 30 minutes suggests that healthy humans can experience exposures to SO_2 up to 10 ppm with only transitory effects on pulmonary function, even under challenging conditions involving hyperventilation, mouth-only exposure, and heavy exercise. Transitory effects may be observed at concentrations as low as 0.75 ppm.

For exposures up to 30 minutes, asthmatics appear to demonstrate pulmonary effects at lower thresholds (0.1 ppm), although even in this population subgroup the clinical effects are transient and may or may not require intermittent pharmacologic intervention.

Few studies investigated exposures in asthmatics longer than 30 minutes. Those that did reported transitory pulmonary function effects at exposure levels of 0.50 to 1 ppm. The studies investigating healthy subjects at these longer time ranges investigated concentrations between 0.75 and 25 ppm. A concentration-dependent response in discomfort was reported between 1 and 25 ppm. Transitory effects on pulmonary function and nasal mucous flow were reported up to 5 ppm at these longer time ranges.

The weight of evidence for single exposures up to 4 hours and repeated exposures between 3 days and 3 weeks suggests that transitory pulmonary effects might be expected for asthmatics at exposure concentrations between 0.5 and 1 ppm and for healthy humans between 0.75 and 25 ppm, with some evidence for a concentration-dependent response in healthy subjects.

Non-clinical

Of the 93 non-clinical studies investigating respiratory effects, 39 (42%) were ranked high or moderate. These studies looked at a variety of species and health outcomes. In addition there was substantial variation in the concentrations and exposure times investigated. Exposures included single exposures of a few hours or less to several days as well as multiple exposures of a few hours per day for up to 30 days.

The concentrations in studies of animals exposed for ***up to 2 hours*** *ranged between 0.5 ppm and 1000 ppm. For concentrations up to 100 ppm, effects reported were predominantly very mild respiratory effects and changes at the cellular or ciliary level. Above 100 ppm, greater pulmonary effects were in evidence, with indications of changes to the lung. There is evidence of increasing severity of effect with increasing concentration.*

In studies with exposures ***between 2 and 24 hours****, mild respiratory effects and delayed airway reactivity were reported with concentrations up to 40 ppm. Damage to the lungs was reported at concentrations of 800 ppm and 1225 ppm.*

At exposures ***between 1 and 7 days****, slight changes were observed in lung function and in response to virus challenges at concentrations of 0.1 ppm to 34.5 ppm. At the higher concentrations of 100 ppm and 600 ppm, changes to lung structure were reported.*

Five studies investigated exposures ***between 7 and 30 days****. One study reported changes in response to virus challenges with exposures up to 0.1 ppm for 4 weeks. The other four studies reported changes in lung biochemistry and some decrease in pulmonary function at concentrations between 10 and 600 ppm.*

Epidemiology

Of the epidemiology studies and case reports investigating respiratory effects, less than half were ranked moderate. There were no high quality epidemiology studies.
The limitations of these epidemiology studies are similar to those outlined in the mortality section.

Epidemiology studies were divided into two types based on calculation of exposure concentration. One set of studies calculated exposures as increases in ambient concentration above a baseline or average concentration. These studies report results as an increase in outcomes (e.g. hospital admissions for asthma) per increase in ambient concentration. For example, a study might report results as a 1.6% increase in hospital admissions for every 3.5 ppm increase in ambient SO_2 concentration. Another set of studies reported exposure as discrete concentrations, either as average concentrations or a concentration range. These studies might report results as, for example, 7% more admissions during periods of higher pollution.

A weight of evidence evaluation is difficult for the epidemiology studies as the majority of the epidemiology studies were ranked low quality. For the moderate quality studies reporting both types of exposure metric, there was an equal number of studies that found insignificant or no associations between *ambient SO_2 concentration and health outcomes as there were that reported an association.*

Deriving causal associations from environmental epidemiologic studies is difficult for a number of reasons. Exposure misclassification is a major limitation of these studies. An additional limitation involves the classification of outcome. In several cases, the respiratory diseases investigated, particularly COPD and asthma, did not have clear case definitions for the purposes of the study, which could lead to inaccurate or inconsistent diagnoses of the health outcomes.

The challenge of different exposure metrics has been discussed. For those studies looking at increases above a baseline, it should be noted that the baseline concentrations differ for each study. In addition, the time-averaging or time over which exposure was calculated is different between studies, making comparisons difficult. The populations used tended to be small and relatively undefined. Most of the studies endeavored to find correlations between ambient levels of SO_2 and rates of health outcomes. Very few calculated relative risks or similar epidemiological statistics. Statistical significance was not calculated in many studies. For those studies that did report statistically significant results, the lower confidence intervals were often very close to one and there were no associations that would be considered strong (OR >2).

F. Signs and Symptoms

Clinical

Effects observed-healthy subjects

Kulle et al. (1986) observed statistically significant increases in nose and throat irritation after exposing adult subjects to 1 ppm SO_2 for 4 hours/day, 3 days/week for 3 weeks (Study ID ▲ 096).

Speizer and Frank (1966b) exposed healthy male subjects to 15 and 28 ppm SO_2 orally or nasally for ten minutes. They observed coughing during the first few minutes in subjects exposed by mouth. These subjects also reported burning sensations of the throat and substernal area for the first five minutes of exposure. Those subjects exposed through the nose had no chest symptoms and little coughing, although they did report some irritation of the posterior pharynx, which lasted for several minutes (Study ID ◆ 054).

Andersen et al. (1974) examined subjective response, among other symptoms, in healthy adult males exposed to between 1 and 25 ppm SO_2 for 6 hours/day for 3 days. Some discomfort, proportional to SO_2 concentration, was reported by the subjects. Subjects with nasal mucus flow rates that were initially slow experienced the greatest discomfort (Study ID ◆ 063).

Toyama and Nakamura (1964) exposed healthy male subjects to concentrations of 1 to 60 ppm SO_2 for 5 minutes. Subjects reported objectionable odours, irritation of the upper respiratory tract, cough, and unusual sensations in the lungs during the first few minutes of exposure (Study ID ● 053).

Sandstrom et al. (1988) endeavoured to establish standardized procedures for exposure to SO_2 in clinical trials and compared mucosal and airway effects from SO_2 vs. air exposure. They exposed eight healthy subjects to 0.4 to 4 ppm SO_2 for 20 minutes. Subjects reported irritation of the throat and an unpleasant smell (Study ID ● 087).

Effects observed-asthmatic subjects

Gong et al. (1995) examined SO_2-sensitive asthmatics to determine the effects of SO_2 exposure up to 0.5 ppm for 1 hour compared to different intensities of exercise on lung function, asthma symptoms, and stamina. They observed that increasing SO_2 levels had greater negative effects on lung function, symptom scores and stamina ratings than did increasing the intensity of exercise (Study ID ▲ 077).

Balmes et al. (1987) studied the relationship between duration and concentration of SO_2 exposure and bronchoconstriction in male and female adult asthmatics. The subjects were exposed to 0.5 or 1 ppm SO_2 for 1, 3 and 5 minutes. Of the eight subjects, two developed substantial chest tightness after inhalation of 1 ppm for 1 minute. Seven of the eight subjects developed wheezing and chest tightness after inhaling 0.5 ppm for 3 and 5 minutes (Study ID ◆ 064).

Hackney et al. (1984) exposed young adult asthmatics to 0.75 ppm SO_2 for three hours. The subjects vigorously exercised for the first 10 minutes of exposure and rested for the balance of the exposure time. General asthmatic symptoms were increased post-exercise relative to pre-exposure levels, but these effects had returned to pre-exposure levels by one hour post-exercise (Study ID ◆ 079).

Koenig et al. (1981) exposed asthmatic adolescent subjects to 1 ppm SO_2 for 30 minutes at rest and 10 minutes during exercise. Three subjects reported shortness of breath and five reported wheezing (Study ID ◆ 041).

Roger et al. (1985) exposed young asthmatic male subjects to levels of SO_2 of 1 ppm for 75 minutes/week for 4 weeks. The subjects reported shortness of breath and chest discomfort after 10 minutes of exposure to 1 ppm. A trend towards increased wheezing, deep breathing discomfort, and cough was also observed (Study ID ● 087).

Effects observed-healthy and asthmatic subjects

Witek et al. (1985) investigated subjective responses to concentrations of SO_2 less than 1 ppm for 40 minutes in healthy and asthmatic subjects. Asthmatics reported chest tightness, wheezing, dyspnea and cough, while the healthy subjects reported taste and odour complaints. There was an increase in the number and severity of the complaints associated with increasing concentration (Study ID ◆ 093).

No effects observed

Bailey et al. (1982) exposed twenty-four young asthmatic adults to 0, 0.25, and 0.5 ppm SO_2 for one hour. No significant symptoms were observed after exposure (Study ID ▲ 075).

Kagawa (1983) observed no symptoms in seven healthy male subjects exposed to 0.15 ppm SO_2 for 2 hours (Study ID ◆ 072).

Linn et al. (1985b) exposed twenty-four adult subjects with chronic obstructive pulmonary disease to 0, 0.4 and 0.8 ppm SO_2 for 1 hour. There were no significant changes in symptom reporting between the pre- and postexposure periods (Study ID ◆ 101).

Eye symptoms

Douglas and Coe (1987) applied concentrations of SO_2 ranging from 3 to 60 ppm SO_2 to the eyes of subjects for

15 seconds using goggles and to the lungs for 10 breaths, via a mouthpiece. These exposure conditions, particularly for the eyes, were quite extreme. Observed eye response included a dose-dependent increase in tear production with exposure, which returned to baseline within 15 minutes post-exposure. The threshold concentrations at which symptoms were first seen or reported were observed. The threshold concentration for eye effects was observed to be 5 ppm, while that for the lung was 1 ppm (Study ID ● 121).

Non-clinical studies

Haider et al. (1981) exposed guinea pigs to 10 ppm SO_2 for 1 hour/day for 21 days to observe any changes in brain lipid chemistry. They observed signs of nasopharyngitis, somnolence, staggering, itching, preening, and skin and eye-irritation (Study ID ▲ 159).

Johnson et al. (1972) exposed male mice to 40 ppm SO_2 for 4 to 11 days and observed depressed feed and water intake, depressed body weight and O_2 consumption, and upper respiratory damages upon initial exposure. Immediately upon cessation of exposure, recovery of body weight began and O_2 consumption was normal by 32 to 34 days post-exposure (Study ID ◆ 261).

Matsumura (1970a, 1970b) exposed guinea pigs to 20, 60, 180 and 300 ppm SO_2 for 30 minutes in one experiment (Study ID ● 142) and 400 ppm for 30 minutes in another (Study ID ● 143). No signs of irritation were seen at concentrations lower than 300 ppm.

Epidemiology studies

Donoghue and Thomas (1999) examined the relationship between atmospheric SO_2 concentrations and hospital presentation for asthma in a town in which both a copper smelter and a lead smelter are major producers of SO_2. Over a three-year period, they observed no association between peak SO_2 concentrations up to 3300 ppb and hospital presentations or admissions for asthma, wheeze, or shortness of breath (Study ID ◆ 007).

Cohen et al. (1974) conducted a telephone survey of irritative symptoms during publicized and unpublicized elevated air pollution events and during a time of low air pollution. They observed increases in reported eye and throat irritation, chest discomfort, shortness of breath, restricted activity, and medical visits in adults during the two high pollution episodes compared to the low pollution time period. Atmospheric SO_2 concentrations were measured at between 0.01 and 0.15 ppm (Study ID ◆ 011).

Several studies investigated general hospital admissions or school absences without specific health outcomes. Xu et al. (1995a,b) conducted a time-series analysis of daily hospital visits and air pollution data. A 38 ppb increase in SO_2 was associated with internal medicine and pediatric outpatient visits, and emergency room visits. SO_2 was found to be a highly significant predictor of total and nonsurgery outpatient visits. Information on monitoring station location was not reported. In addition, only patients able to pay the registration fee would go to the hospital, suggesting some bias is likely (Study ID ● 424, 474).

Park et al. (2002) found that exposure to SO_2 in the range of 2.68 to 28.11 ppb) was associated with illness-related absences from school among elementary school students. The specific type of illness was not reported. There was also a significant protective association between SO_2 and non-illness related absences. Again, specific reasons for these absences were not recorded. The teachers determined whether the absences were related to illness or not for the purposes of the study (Study ID ● 429).

Case reports

Several case reports of accidental exposure to very high levels of SO_2 by fewer than 10 individuals have been reported. Rabinovitch et al. (1989) presented a two-year follow-up of two miners who had been exposed to high concentrations of SO_2 after a mine explosion. Among various airway effects, the individuals reported markedly reduced exercise tolerance (Study ID ◆ 272).

Harkonen et al. (1983) report on a four-year follow-up of seven men who were accidentally exposed to SO_2 in a pyrite dust explosion. Immediately following these high levels of exposure, the men exhibited thoracic pain, coughing, conjunctival irritation and in some cases, corneal erosion (Study ID ● 021).

Galea (1964) reported a case of a 35-year old man who accidentally inhaled high levels of SO_2. Ten days after exposure the patient reported dry, irritable, tiresome cough, dyspnea, and copious amounts of mucus (Study ID ● 271).

Woodford et al. (1979) describe the case of a previously healthy young man who reported symptoms of burning and tearing eyes, rhinorrhea, cough and almost passing out after a brief exposure to a high concentration of SO_2 (Study ID ● 269).

Charan et al. (1979) reported the symptoms of three survivors of an industrial accident in which five men were exposed to high concentrations of SO_2. The three survivors reported irritation and soreness of the eyes, nose, and throat, tightness in the chest, and intense dyspnea. An eye examination showed severe conjunctivitis and superficial corneal burns (Study ID ● 270).

Summary

Clinical

Several studies reported signs and symptoms as observations concurrent to investigation of other effects. Healthy subjects reported nose and throat irritation, taste and odour complaints, and discomfort during single exposures (15 and 28 ppm for 10 minutes or to less than 1 ppm for 40 minutes) as well as during multiple exposures (1 ppm for 4 hours/day, 3 days/week for 3 weeks and 1 to 25 ppm for 6 hours/day for 3 consecutive days). Some coughing was observed in the healthy subjects during forced mouth breathing. Asthmatic subjects reported chest tightness, shortness of breath, wheezing, asthma symptoms, dyspnea and cough during single exposure both with and without exercise. Concentrations ranged from 0.5 to 1 ppm and lasted between 3 minutes and 3 hours. No symptoms were reported in asthmatics after exposure to 0.5 ppm for one hour, subjects with COPD exposed to 0.8 ppm for one hour, or healthy subjects exposed to 0.15 ppm for 2 hours.

Non-clinical

Few non-clinical studies reported irritative symptoms as a result of SO_2 exposure. Itching, preening, somnolence, and eye- irritation were observed in guinea pigs exposed to 10 ppm for 1 hour/day for 21 days. Depressed feed *and water intake and decreased body weight and oxygen consumption were observed in male mice exposed to 40 ppm for 4 to 11 days. Recovery of body weight and oxygen consumption began immediately after cessation of exposure.*

Epidemiology

Shortcomings in epidemiology studies and case-reports have been detailed in the mortality and respiratory summaries of this report. The same limitations apply to the few moderate epidemiology studies and case-reports reporting general signs and symptoms. Responders in a telephone survey during elevated air pollution events with ambient SO_2 levels up to 0.15 ppm reported increased eye and throat irritation, chest discomfort, shortness of breath, and restricted activity. Two miners exposed to very high concentrations in a mine explosion reported reduced exercise tolerance two years after the accident. No association was reported between ambient SO_2 concentrations up to 3.3 ppm and hospital admissions for asthma, wheeze, or shortness of breath.

G. Cardiovascular System

Non-clinical studies were most numerous, although clinical and epidemiology studies were also available. However, many of the studies were judged to be of questionable quality and the information obtained not very reliable or relevant.

Clinical studies

Only two clinical studies dealt with cardiovascular effects. Tunnicliffe et al. (2001) exposed 12 normal and 12 asthmatic male and female adult humans to 200 ppb SO_2 (to mimic likely air pollution levels) for 1 hour and recorded the electrocardiograms. No significant differences in maximum or minimum heart rates were seen in either group. In normal subjects, total power, high frequency power and low frequency power appeared higher with SO_2 exposure. These indices appeared lower in the asthmatic group upon SO_2 exposure. However, the only statistically significant result was the difference in total power found in air *vs.* SO_2 in normal subjects. This lone result is weak evidence for the authors' conclusion that "SO_2 exposures at concentrations which are frequently encountered during air pollution episodes can influence the autonomic nervous system" (Study ID ◆ 071).

Amdur et al. (1953) observed no statistically significant effects on pulse-rate in subjects exposed to 1 to 8 ppm SO_2 for 10 minutes (Study ID ● 032).

Non-clinical studies

Several studies investigated changes in heart rate with SO_2 exposure. A single high quality study found no differences in heart rate or blood pressure in chickens exposed to 100 ppm SO_2 for 1 hour (Fedde and Kuhlmann, 1979). A statistically significant increase in heart rate was observed upon exposure to 5000 ppm for one hour. Most of the chickens exposed at this level died (Study ID ▲ 183).

Wang et al. (1996) observed a mild decrease in heart rate in Sprague-Dawley rats with exposure for two tidal breaths to air containing 5000 ppm SO_2. This decrease lasted for 3 to 8 breaths before returning to control levels. No increase in blood pressure was observed (Study ID ◆ 211).

Callanan et al. (1974) observed an increase in blood pressure and heart rate in geese after inhalation of 100 to 400 ppm of SO_2 for 1 to 3 minutes. The same

study found striking differences between geese and mammals in terms of response to inhaled SO_2 (Study ID ◆ 233).
Drew et al. (1983) exposed two lines of Dahl rats to SO_2 at 50 ppm for 6 hours per day, 5 days a week for 6 weeks. One line of rats was resistant to salt-induced hypertension; the other was not. In the rats resistant to hypertension, a slight but consistent decrease in blood pressure was observed with exposure to SO_2. In the rats not resistant to hypertension, an increase in blood pressure was observed in the SO_2-exposed animals versus the air-exposed animals. These observations disappeared after the last SO_2 exposure (Study ID ● 241).

Langley-Evans et al. (1996) observed depleted glutathione levels in the hearts of rats exposed to levels of SO_2 as low as 5 ppm and as high as 100 ppm for 5 hours/day, for 7 to 28 days (Study ID ◆ 251).
Haider (1985) observed elevated levels of cholesterol, total lipids and phospholipids in guinea pig hearts and decreased concentration of gangliosides after exposure to 10 ppm for 1 hour a day for 30 days (Study ID ◆ 163).
Rana et al. (1979) observed decreased lipid levels and increased moisture content in squirrel hearts after exposure to 500 ppm SO_2 for 4 minutes (Study ID ● 147).
Balchum et al. (1960) observed a low, uniform concentration of $^{35}SO_2$ in the heart muscle of dogs after inhalation of 1.8 to 148 ppm $^{35}SO_2$ for 30 to 40 minutes (Study ID ● 237).

Epidemiology studies
Wong et al. (2002) reported significant positive associations of similar size between an incremental 4 ppb increase of SO_2 (baseline concentrations 6.8±4.7 ppb) and daily admissions for cardiac diseases in Hong Kong and London, England. No information is reported on the number of monitoring stations and their location (Study ID ◆ 423).
Sunyer et al. (2002) observed a significant increase in daily numbers of all cardiovascular admissions except stroke and particularly ischemic heart disease with a same-day increased in daily SO_2 concentrations (range: 1.9-8 ppb). However, not all cities involved in this study were able to provide complete exposure and outcome data. Confounding factors were not considered (Study ID ● 459).
Morris et al. (1995) reported inconsistent results for the association between an increase of 0.05 ppm SO_2 and hospital admissions for congestive heart failure. Only two of seven US cities had statistically significant associations: New York with the highest average SO_2 levels, and Los Angeles with the lowest. The results and discussion section focus on CO. There is limited information on monitoring and many confounding factors (Study ID ● 387).

No association observed
In a study on the effects of atmospheric SO_2 pollution on mortality, Derriennic et al. (1989) compared the populations of two French cities with average SO_2 concentrations of 25 and 19 ppb. No statistical association was found between SO_2 pollution and cardiovascular deaths. However, because of the difficulties of measuring SO_2 exposure and the potential for misclassification of cause of death, these results are inconclusive (Study ID ● 002).
Ponka and Virtanen (1996a) reported no significant associations between SO_2 (range: 0.08-36 ppb) and hospital admissions for ischemic cardiac and

cerebrovascular diseases. Regression results for SO_2 are not reported. Some confounding factors were considered (Study ID ● 388).

Peters et al. (2000) reported no association between implanted cardioverter defibrillator discharges and SO_2 concentrations (mean 0.007 ppm) in Massachusetts. Defibrillator discharge was used a s a surrogate measure for cardiac arrhythmia. Exposure assessment was a weakness as only one monitor collected data for a large area of eastern Massachusetts. Statistical significance was not reported (Study ID ● 441).

Summary:

Clinical studies:
Only one moderate quality study was identified as investigating cardiovascular effects. This study reported weak evidence of a difference in total power recorded in an electrocardiogram after exposure to 0.2 ppm for 1 hour.

Non-clinical studies:
A high quality study reported an increase in the heart rate of chickens with exposure to 5000 ppm for 1 hour, but no effect on heart rate or blood pressure at exposure to 100 ppm for 1 hour. Two moderate studies also investigated effects on heart rate and blood pressure. Rats exhibited decreased heart rate after two tidal breaths of 5000 ppm. Geese exhibited increased blood pressure and heart rate with 1 to 3 minutes of exposure to 100 to 400 ppm. Multiple exposure designs identified decreased glutathione in the hearts of rats (5 to 100 ppm for 5 hours a day for 28 days) and an increase in cholesterol, total lipids, and phospholipids in guinea pig hearts (10 ppm for 1 hour/day for 30 days). The clinical significant of these results is unclear.

Epidemiology studies:
In the lone moderate study in this category, a small but significant association was reported between daily admission for cardiac disease in London England and Hong Kong and an incremental increase of 4 ppb in ambient SO_2 concentrations (baseline concentrations 6.8±4.7 ppb). The limitations previously identified for epidemiology studies apply to this study. In particular, exposure assessment was a major limitation and the study was rated "moderate-to-low".

H. Eye

Clinical studies

In an investigation of the effects of SO_2 and respirable carbon aerosol on 20 healthy, non-smoking adult subjects, Kulle et al. (1986) observed no adverse effects on the eye with exposure to 1 ppm SO_2 alone, 4 hours per day, 3 days per week for 3 weeks (Study ID ▲ 096). Two studies observed increases in tear production with exposure to SO_2. Coe and Douglas (1982) exposed six subjects to 50 ppm SO_2 for 5 minutes. None of the subjects reported subjective sensations of eye irritation during or after this exposure period (Study ID ● 065).

In contrast, Douglas and Coe (1987) exposed subjects to levels of SO_2 varying from 3 to 60 ppm for 15 seconds. The threshold for tear production was found to be 5 ppm (Study ID ● 121).

Non-clinical studies

Only one non-clinical study reported any effects of SO_2 exposure on the eyes

(Haider et al., 1981). In this study eye effects were not the main focus and are mentioned briefly amongst other signs and symptoms of SO_2 exposure as follows: "exposure to guinea pigs to SO_2 lead to signs of….eye irritation…". Guinea pigs were exposed to 10 ppm, one hour per day for 21 days (Study ID ▲ 159).

Epidemiology studies

Cohen et al. (1974) carried out a telephone survey to investigate the reporting of irritative symptoms during well-publicized and unpublicized periods of moderately elevated air pollution and a period of low pollution. Peak hourly SO_2 concentrations ranged between 0.01 and 0.15 ppm for the low and elevated pollution events. Significant increases in eye irritation were observed during both elevated pollution episodes compared to the control (low pollution) period. There was no difference in the reporting of eye irritation between the publicized and unpublicized episodes (Study ID ◆ 011).

Complaints of irritative effects prompted a Health Hazard Evaluation of Portland Cement plants to assess whether SO_2 exposure was the genesis of the problems (NIOSH, 1984). SO_2 levels at the plants were measured to be 0.2 to 1.8 ppm. The question of whether SO_2 exposure caused the eye irritation was undetermined (Study ID ● 267).

Harkonen et al. (1983) followed 7 men unintentionally exposed to "high levels" of SO_2 for 20 to 45 minutes during an explosion in a pyrite mine. The levels of SO_2 exposure were not determined. Immediately after exposure, conjunctival irritation in all cases and corneal erosion in some cases were observed (Study ID ● 021).

Woodford et al. (1979) describe the case of a healthy young man accidentally exposed to a high concentration of SO_2 for 15 to 20 minutes. The actual SO_2 concentration was not determined. The subject reported symptoms of burning and tearing eyes after exposure. (Study ID ● 269).

These last two studies are of limited use in determining the effects of low dose exposure to SO_2, being case-reports rather than epidemiologic or clinical studies. In addition, SO_2 exposure concentrations were not measured. In all epidemiology studies, the issue of SO_2 exposure determination is not well addressed.

Summary:

SO_2 is generally acknowledged to have irritant effects on the eye. However, very few studies fitting the criteria for this report (e.g. peer-reviewed, scientific literature) report eye effects and none of the studies reviewed specifically investigated eye effects. Some studies with a focus on other health endpoints reported eye effects as a peripheral observation.

Clinical studies:

Of the three studies reporting eye effects, one was rated of high quality while the other two were low quality. The high quality study reported no adverse effects on the eye with exposure to 1 ppm for 4 hours/day, 3 days/week for 3 weeks.

Non-clinical studies:

The single non-clinical study reporting eye effects was ranked high quality. Eye effects were not a major focus of this study. However, the study reports that exposure of guinea pigs to 10 ppm for 1 hour/day for 12 days leads to signs of eye irritation.

Epidemiology studies:

Only one of four epidemiology studies mentioning eye effects was ranked moderate quality. This study reported that increases in eye irritation were observed during elevated pollution events with ambient levels up to 0.15 ppm.

I. Gastrointestinal System

No studies clinical or epidemiology were found that investigated or reported effects to the gastrointestinal system as a result of acute SO_2 exposure.

Non-clinical studies

Meng et al. (2003) suggest that inhalation of SO_2 (8.4±0.8, 21±1, and 43±3 ppm) increased levels of lipid peroxidation in stomachs and intestines of male and female Kunming albino mice. These results suggest a toxicological role of SO_2 inhalation on these organs in mice. Confidence intervals were not reported, but Good Laboratory Practice guidelines were generally followed (Study ID ◆ 460).

J. General Biochemical Effects

There is slight human and animal evidence for the potential to use SO_2 metabolites as a possible biomarker of exposure to SO_2. There is also some evidence of various other biochemical effects. However, the clinical relevance of these effects is unclear. The confidence level of many of the studies was low, limiting the reliability and utility of the results.

Clinical studies

Trenga et al. (1999) tested 47 adult asthmatics for characteristics associated with SO_2 sensitivity. They found correlations between plasma beta-carotene concentrations and peak expiratory flow values, ascorbate concentrations and baseline pulmonary function indices, as well as with high-density lipoprotein concentrations and forced expiratory flow values. There were no correlations between plasma antioxidant nutrient concentrations and sensitivity to inhaled SO_2 (Study ID ◆ 055).

Kienast et al. (1994b) reported a dose-dependent stimulation of reactive oxygen intermediates (ROI) after 30 and 60 minutes of exposure to SO_2 at levels of 0.3 to 1.5 ppm. However, the study authors report that there is no conclusive evidence as to the measured amount of ROI that is sufficient to induce clinically relevant pulmonary fibrosis, so the clinical significance of these findings is uncertain (Study ID ◆ 312).

Gunnison and Palmes (1974) investigated the possible production of S-sulfonate in human plasma as a function of exposure to SO_2. After subjecting humans to controlled SO_2 exposures of 0, 0.3, 1, 3, and 6 ppm continuously for up to 120 hours, they determined that plasma levels of S-sulfonate showed a positive correlation with atmospheric sulfur dioxide. However, the weaknesses of this study limit confidence in the results. Little detail was available on the characteristics of the study volunteers, the monitoring methods, and the method of exposure (Study ID ● 112).

Grote and Thews (1973) observed that the amount of SO_2 that can dissolve in human blood with base excess values between +10 and –15 mEq/L and normal hemoglobin increases with increasing blood O_2 or CO_2 and decreasing blood pH. The experimental detail was lacking in terms of SO_2 exposure delivery and dose measurement. Recruitment methods and characteristics of the volunteers such as age and pre-trial health were not reported (Study ID ● 265).

Yokoyama et al. (1971) reported that whole blood levels of $^{35}SO_2$ rose steadily with exposure to 50 ppm. The exposure duration was not clearly stated in the study, nor are many important experimental details included in the report. The clinical and toxicological significance of these results are unclear (Study ID ● 373).

Non-clinical studies

Guinea pigs

Etlik et al. (1995) found higher methemoglobin and sulfhemoglobin, as well as lipoperoxidation and osmotic fragility in guinea pigs exposed to 10 ppm SO_2 by inhalation for 1 hour per day for 30 days (Study ID ◆ 236).

Matsumura (1970) observed hemaglutination in five of ten guinea pigs exposed to 330 ppm SO_2 by inhalation for 30 minutes. Age and sec of animals were not specified which is a consideration when only one half of the animals displayed a response (Study ID ● 142).

In a general exploration of the biological effects of SO_2 exposures on guinea pigs, Lee and Danner (1966) observed increased hemoglobin at all concentrations between 6 and 310 ppm and blood inorganic sulfur concentrations above 19 ppm for a 60-minute exposure. Strain, sex, and pre-trial health, feed and test conditions were not reported. There was a single animal for each dose and statistical significance was not reported (Study ID ● 254).

Rats

Lovati et al. (1996) investigated the effects of SO_2 exposure on serum lipids/lipoproteins and on glucose metabolism in rat models of hypercholesterolemia and diabetes. In normal animals fed either a standard or a cholesterol-enriched diet, they observed a dose-dependent increase in plasma triglycerides and a significant reduction in HDL cholesterol levels. A reduction of plasma triglycerides and an increase in plasma HDL cholesterol was observed in diabetic animals upon exposure to 5 or 10 ppm SO_2 continuously for 15 days (Study ID ▲ 152).

Jonek et al. (1976) traced the uptake of SO_2 into the bodies of Wistar rats. Rats were exposed to $^{35}SO_2$ in air by inhalation. The highest radioactivity in blood serum was observed 2 hours after exposure. The radioactivity was still high 24 hours after exposure, but decreased in the following days (Study ID ◆ 151).

Baskurt (1988) exposed male Swiss albino rats to 0.87 ppm SO_2 for 24 hours and observed higher hematocrit and sulfhemoglobin values in the exposed animals compared to the controls. They observed that whole blood and packed cell viscosities were lower in the rats exposed to SO_2 than in the controls. There were no differences in the plasma viscosities between exposed and control animals (Study ID ◆ 192).

Azoulay et al. (1980) found no differences in blood variables or hemoglobin affinity in rats exposed to 2 ppm SO_2 continuously for 1 to 49 days compared to control animals for 1 hour/day for 30 days (Study ID ◆ 225).

Gause and Barker (1978) investigated the uptake of SO_2 in the Sprague-

Dawley rat during exposures of 5 to 20 ppm continuously for 7 days and observed that approximately 10% of the SO_2 inhaled is found in the plasma or serum within the first 30 minutes of exposure. Exposure assessment was not well defined and information on the experimental protocol is lacking (Study ID ● 193).

Baskurt et al. (1990) observed no significant differences between the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC between control rats and rats treated with 1 ppm SO_2. Erythrocyte deformability indexes of the SO_2 treated animals were significantly higher than controls. However, Good Laboratory Practice guidelines were not followed in this study, which has substantial weaknesses in design and reporting (Study ID ● 302).

Mice

Vanjonack and Johnson (1974) exposed mice to 40 ppm SO_2 for times between 0.5 and 24 hours and observed a statistically significant time-dependent decrease in plasma thyroxine levels at exposure times of 12 and 24 hours. There was also a statistically significant time-dependent increase in plasma glucocorticoids at 1 and 12 hours (Study ID ◆ 212).

Meng et al. (2002) observed increased frequencies of polychromatic erythrocyte micronuclei formation (MNPCE) in mouse bone marrow with increased doses of SO_2 from 5 to 32 ppm. The authors state that these are preliminary results and clinical significance is unclear although the study authors suggest that SO_2 may be a clastogenic and genotoxic agent in mice (Study ID ● 380).

Rabbits

Gunnison and Benton (1971) did not detect free sulfite in the plasma of rabbits immediately following exposure to 23.5 ppm SO_2 continuously for 14 or 62 hours. However, elevated levels of plasma and serum S-sulfonate were observed. Animal characteristics and treatment were not specified, nor were pre-test and acclimation conditions (Study ID ● 222).

Chickens

Fedde and Kuhlman (1978) exposed male chickens to concentrations of SO_2 up to 5000 ppm for 60 minutes by inhalation, either through tracheal cannulae or with intact respiratory systems. They observed no changes in arterial blood gases and pH at 100 ppm; however, there were statistically significant decreases in blood pH and increases in blood CO_2 at 5000 ppm with both methods of inhalation. In addition, statistically significant decreases in blood O_2 were observed in those birds with intact respiratory systems (Study ID ▲ 183).

Epidemiology studies

No epidemiology studies investigated this outcome for SO_2 exposure, specifically.

Summary:

Clinical

Two moderate quality studies were identified. In one study, no association was observed between plasma antioxidant nutrient concentrations and sensitivity to inhaled SO_2. In the other study, a dose-dependent stimulus of *reactive oxygen intermediate (ROI) was reported with exposure to concentrations between 0.3 and 1.5 ppm for 30 to 60 minutes. However, it is unclear how much ROI stimulation is required to induce clinically relevant pulmonary fibrosis.*

Non-clinical

Two high quality studies were identified. One investigated the effects of SO2 exposure on serum lipids and lipoproteins and glucose metabolism in diabetic and normal rats. With continuous exposures of 5 and 10 ppm for 15 days, increases in plasma triglycerides and decreases in plasma HDL cholesterol were reported in the healthy rats. Increases in plasma triglycerides and increases in plasma HDL cholesterol were reported in the diabetic rats. The other high quality study investigated responses of chickens to high levels of SO_2 (5000 ppm for 1 hour). Decreased blood pH and increased blood CO_2 were observed. Moderate studies reported increased sulfhemoglobin values, and lower whole blood and packed cell viscosity, but no differences in plasma viscosity (0.87 ppm for 24 hours in rats), and a time-dependent decrease in plasma thyroxine levels in mice exposed to 40 ppm for 12 to 24 hours. One study reported no differences in blood variables or hemoglobin affinity in rats exposed to 2 ppm continuously for 1 to 49 days.

Epidemiology

No epidemiology studies were identified that fit the criteria for inclusion.

K. Immunological System

There is some evidence that SO_2 influences the immunological system. Various effects and potential mechanisms of action have been investigated, making verification of effects and identification of SO_2 concentrations difficult.

Clinical Studies

Winterton et al. (2001) exposed asthmatic subjects to 0.5 ppm SO_2 for 10 minutes and analyzed buccal cell samples for genetic polymorphisms. They found that increased response to SO_2 was associated with wild-type allele of the TNF-alpha promoter polymorphism, but not with other polymorphisms. They concluded that the mechanisms of asthmatic sensitivity may be associated with this wild-type allele (Study ID ◆ 035).

Anderson et al. (1977) experimentally induced rhinovirus infection in two groups of subjects. One group was exposed to SO_2 at 5 ppm for 4 hours while the other group (controls) was exposed to pollution-free air under the same conditions. There was no difference in the number of subjects who developed colds, but there was a 50% decrease in nasal mucus flow rate in the SO_2-exposed group compared to the control group. The SO_2-exposed group also had fewer symptoms and less but more persistent virus shedding than the control group. There were no differences in antibody response between the two groups (Study ID ◆ 048).

Sandstrom et al. (1989) used bronchoalveolar lavage to investigate the effect of SO_2 on the human lung. Twenty-four hours after exposure to 4 ppm SO_2 for 20 minutes, there was an increase in alveolar macrophage activity. Twenty-four hours after exposure to 8 ppm SO_2 for 20 minutes, a greater increase in alveolar macrophage activity was observed. Seventy-two hours after exposure, activity had returned to pre-exposure levels (Study ID ◆ 083).

Sandstrom et al. (1989) performed bronchoalveolar lavage on eight subjects 4, 8, 24 and 72 hours after exposure to 4, 5, 8, or 11 ppm SO_2 for 20 minutes. They found a significant increase in lysozyme positive and alveolar macrophages/monocytes at all concentrations. After exposure to 8 and 11 ppm SO_2, the total number of alveolar macrophages/monocytes was significantly increased. Increases in the total number of mast cells were seen 24 hours after exposure to 5 ppm SO_2 and above (Study ID ● 091).

Koenig et al. (1987) investigated the effect of albuterol in preventing SO_2-induced bronchoconstriction. Their results suggest that the mechanism of action for SO_2-induced bronchoconstriction involves mast cell degranulation or the adrenergic nervous system (Study ID ◆ 103).

Sheppard et al. (1981a) investigated the mechanism of action of SO_2 by exposing asthmatic subjects to 0.5 or 1.0 ppm SO_2 and cromolyn or lactose for 10 minutes while exercising. Their results suggest that SO_2 may induce bronchoconstriction by stimulating the release of mediators from mast cells (Study ID ● 058).

Non-clinical studies

Azoulay-Depuis et al. (1982) investigated the effect of SO_2 exposure on resistance to respiratory infection by exposing female mice to 10 ppm SO_2 for up to 3 weeks. There was a significant increase in mortality in the groups of mice exposed to SO_2 for 7 or more days compared to controls. There was a decrease in mean survival time for mice exposed to all levels of SO_2 (Study ID ▲ 172).

Ukai (1977) investigated the effect of exposure to low levels (0.03 to 0.1 ppm) of SO_2 on the pathogenesis of influenza virus infection in mice. In mice exposed to both SO_2 and the virus, antibodies to the virus developed more rapidly than in those mice exposed to virus alone. In addition, mice exposed to SO_2 but not the virus showed an increase in the number of goblet cells in nasal epithelial cells. These observations suggest that SO_2 alters the nasal mucus membranes, eliminating a major defensive barrier against disease and subsequently resulting in increased severity of influenza infection (Study ID ◆ 207).

Fairchild (1977) investigated the effects of SO_2 on the growth of influenza virus in the nasal epithelia of mice. Exposure to 6 ppm SO_2 for 7 days caused partial inhibition of influenza virus growth in the nasal epithelium and no propagation in the lungs (Study ID ◆ 238).

Trimpe et al. (1986) observed that there was no difference in the rate of clearance of Listeria monocytogenes bacteria from hamsters after exposure to 27 ppm SO_2. Time of SO_2 exposure, either prior to or simultaneous to bacteria exposure, did not affect the clearance rates (Study ID ● 134).

Park et al. (2001) found that repeated exposure to low levels of SO_2 (0.1 ppm) might enhance the development of ovalbumin-induced asthmatic reactions in guinea pigs (Study ID ▲ 259).

Riedel et al. (1988) investigated the effect of SO_2 exposure on bronchial sensitization to inhaled antigens in the guinea pig. After exposing the animals to 0.1-16.6 ppm for 8 hours/day for 5 days, they observed a significant increase in ovalbumin-specific antibodies in serum and bronchoalveolar fluid in SO_2-exposed groups compared to controls (Study ID ◆ 133).

Gause and Rowlands (1975) observed a dose-dependent spectral change in labeled human lymphocyte membranes after exposure to inhaled SO_2. They speculate that the changes may indicate the formation of microparticles or microaggregates of membrane protein structures (Study ID ● 201).

Watson and Brain (1980) investigated the extent of particle uptake in normal and SO_2-damaged airway epithelia in mice. They observed that exposure to SO_2 at 250 ppm for 3 hours increased the uptake of iron in airway epithelium (Study ID ◆ 209).
Okuyama et al (1979) observed an increase in the number of macrophages, lymphocytes, plasma cells and neutrophils in the epithelium and lamina propria of chickens exposed to 3.4 to 18.5 ppm for 1 to 14 days (Study ID ● 199).
Norris and Jackson (1989) observed increased response to aerosolized histamine as a result of exposure to 200 ppm SO_2 for 2 hours in dogs. In addition, there was an increase in airway permeability to plasma proteins and an increase in epithelial cell shedding (Study ID ● 146).

Epidemiology studies
In the only epidemiology study in this section, Boezen et al. (1999) investigated factors that could increase children's susceptibility to air pollution. They found that children with bronchial hyperresponsiveness and with high serum concentrations of total IgE (>60 kU/L) were particularly susceptible to air pollution, but not SO_2, specifically (Study ID ◆ 005).

Summary

Clinical
Several studies investigated the mechanisms of action of SO_2 on immunological system functions. Increased alveolar macrophage activity was reported in subjects exposed to 4 and 8 ppm for 20 minutes. One study induced rhinovirus infection in an SO_2-exposed group (5 ppm for 4 hours) and a control group. The number of subjects who developed colds was not different between the two groups. However, the SO_2-exposed group experienced a decrease in nasal mucus flow rate, fewer symptoms, and less but more persistent virus shedding. It has been suggested that mechanisms of asthmatic sensitivity may be associated with a wild-type allele of the TNF-alpha promoter polymorphism or may involve mast cell degranulation.

Non-clinical
Increased mortality and decreased survival time was observed in a group of female mice with respiratory infection exposed to 10 ppm for up to 3 weeks compared to non-exposed controls. Mice exposed to 0.03 to 0.1 ppm and an influenza virus developed antibodies to the virus more rapidly than mice exposed to the virus alone. The literature suggests that SO_2 alters nasal mucus membranes thereby decreasing a defensive barrier to disease and resulting in increased severity of influenza infection. However, another study reported that exposure to 6 ppm for 7 days caused partial inhibition of influenza virus growth in the nasal epithelium and no propagation in the lungs. Studies on guinea pigs suggested

that exposure to low levels of SO_2 (1ppm) might enhance the development of ovalbumin-induced asthmatic reactions and reported a significant increase in ovalbumin-specific antibodies in serum and bronchoalveolar fluid with exposure to 0.1 to 16.6 ppm for 8 hours/day for 5 days. A study on mice exposed to 250 ppm for 3 hours reported an increased uptake of iron in airway epithelium. The clinical significance of many of these studies is unclear and not discussed in the studies themselves.

Epidemiology
One moderate epidemiology study reported that children with bronchial responsiveness and high serum concentrations of total IgE were particularly susceptible to air pollution, but not SO_2 specifically.

L. Kidney and Liver

Clinical studies
No clinical or epidemiology studies were found that investigated or reported liver or kidney health outcomes.

Non-clinical studies
Lovati et al. (1996) observed a dose-related increase in liver weight and an increase of triglycerides in the livers of rats on a standard diet after exposure to 10 ppm SO_2 continuously for 15 days. Diabetic rats showed a decrease in liver weight after exposure to both 5 and 10 ppm SO_2, as well as a dose-dependent decrease in liver triglycerides (Study ID ▲ 152).
Haider (1985) observed depletion of phospholipids, cholesterol and cholesterol/phospholipid ratios (C/P ratios) and decreased lipid peroxidation in guinea pig livers and kidneys after exposure to 10 ppm SO_2 for 1 hour per day for 30 days (Study ID ◆ 163).
Langley-Evans et al. (1996) investigated the detoxification of SO_2 in rats exposed to concentrations from 5 to 100 ppm for 5 hours/day for 7 to 28 days. They observed depleted glutathione levels in the liver and kidney at all concentrations. Glutathione reductase activity was decreased in the liver at 5 ppm. At 50 ppm glutathione S-transferase activity was unaltered in the liver, suggesting an impairment of the sulfitolysis reaction (Study ID ◆ 251).
Balchum et al. (1960) investigated the uptake and distribution of $^{35}SO_2$ in dogs. After inhalation of 1.8 to 148 ppm for 30 to 40 minutes, they observed the highest S^{35} concentration in the trachea, lungs, and lymph nodes with the next highest concentration in the kidneys. A low but uniform concentration was observed in the liver (Study ID ● 237).

Summary:
No clinical or epidemiology studies investigating or reporting liver or kidney effects and fitting the criteria were identified for this review.

Non-clinical
Increases in liver weight and triglycerides in the livers of healthy rats exposed to 10 ppm continuously for 15 days were observed in a high quality study. The same study reported decreased liver weight and a dose-dependent decrease in liver triglycerides in diabetic rats after exposure to 5 or 10 ppm continuously for 15 days. Depletion of phospholipids, cholesterol, cholesterol/phospholipid ratios and lipid peroxidation in guinea pig livers was reported after exposure to 10 ppm for 1 hour/day for 30 days. Glutathione

reductase activity was decreased in rat livers at 5 ppm for 5 hours/day for 7 to 28 days. In addition, glutathione levels in the liver and kidney were reduced at concentrations between 5 and 100 ppm for the same exposure protocol.

M. Metabolic Systems

Clinical

There were no clinical or epidemiology studies investigating the metabolic effect of SO_2 exposure.

Non-Clinical

Johnson et al. (1972) found that continuous exposure of mice to 40 ppm SO_2 for 4 to 11 days depressed metabolism as measured by O_2 consumption. Oxygen consumption returned to normal by 32 to 34 days post-exposure (Study ID ◆ 261).

Meng (2003) reported a statistically significant decrease in Cu, Zn-superoxide dismutase in the brain, lung, stomach, and intestine of male mice and in the lung, heart, liver intestine and kidney in female mice after exposure to 20 ppm SO_2 for 6 hours a day for 7 days. Significantly decreased activities of Se-dependent glutathione peroxidase were observed in all organs of mice of both sexes and a significant decrease of catalase activity in livers from both sexes of mice. The clinical significance was not clear (Study ID ◆ 381).

Langley-Evans et al. (1996) observed varied levels of glutathione, as well as varied enzyme activity in the lung, liver, heart and kidney of Wistar rats exposed to levels of SO_2 between 5 and 100 ppm for 5 hours/day for 7 to 28 days. They concluded that SO_2 is a potential glutathione-depleting agent (Study ID ◆ 251).

Leung et al. (1985) found that glutathione S-sulfonate, a metabolite of SO_2 in the body, acts as an inhibitor of glutathione S-transferase in rat livers and lungs (Study ID ● 273).

Lipid metabolism

Lovati et al. (1996) evaluated the effects of 15 days of continuous SO_2 exposure at 5 and 10 ppm on the lipid and carbohydrate metabolism of rats with and without hypercholesterolemia and diabetes. They observed a dose-dependent increase in plasma triglycerides at 10 ppm SO_2 and a reduction of HDL cholesterol levels. Conversely, the same concentration resulted in a decrease of plasma and liver triglyceride levels and an increase in plasma HDL cholesterol in diabetic rats. The results of this study suggest that SO_2 exposure can modify major lipid and glycemic indices (Study ID ▲ 152).

In guinea pigs exposed to 10 ppm SO_2 for 1 hour per day for 30 days, Haider (1985) investigated the effects of SO_2 on lipid metabolism in guinea pig organs. They observed variations in the concentrations of phospholipids, total lipids, cholesterol, gangliosides and C/P ratio in the liver, heart, lungs and kidney. Also observed were an increased in the rate of malonaldialdehyde formation and lipid peroxidation (Study ID ◆ 163).

Summary

Clinical and Epidemiology

No human clinical or epidemiology studies were identified that investigated this health outcome and fit the criteria.

Non-clinical

Continuous exposure of mice to 40 ppm for 4 to 11 days was reported to depress metabolism as measured by oxygen consumption. Decreased enzyme activity was observed in mice (20 ppm for 6 hours/day for 7 days) and rats (5 to 10 ppm for 5 hours/day for 7 to 28 days). Clinical significance of these observations was not discussed and is unclear.

Changes in lipid metabolism were reported in rats (continuous exposure to 5 and 10 ppm for 15 days) and guinea pigs (20 ppm for 1 hour/day for 30 days).

N. Nervous System

No clinical or epidemiology studies that fit the criteria were identified in this category.

Non-clinical

Behavioural

Petruzzi et al. (1996) observed acute and subacute behavioural changes in male and female mice exposed to 5, 12, and 30 ppm of near continuous exposure to SO_2 for 24 days. Changed behaviours included rearing, social interactions, grooming, digging and chamber-crossing (Study ID ▲ 214).

Fiore et al. (1998) exposed CD-1 mice to 5, 12, and 30 ppm SO_2 prenatally (first 14 days of pregnancy) and investigated changes in aggressive behaviour at adulthood. Changed behaviours included reduced tail rattling, freezing, social investigation and defensive postures. A 20-minute aggressive encounter was set up by pairing an exposed subject with an unexposed CD-1 male opponent of the same age, body weight, and isolation condition as the exposed subjects. Offensive and attack behaviours were unchanged (Study ID ◆ 217).

Biochemical

Haider et al. (1981) investigated the effects of SO_2 exposure at 10 ppm, 1 hour per day for 21 days on guinea pig brain lipids, lipid peroxidation and lipase activity. They found a significant depletion of total lipids and free fatty acids in all brain regions. Phospholipid, cholesterol, esterified fatty acid concentrations and the rates of lipid peroxidation and lipase activity were affected differently in different parts of the brain (Study ID ▲ 159). Haider et al. (1982) investigated the effects of SO_2 exposure at 10 ppm, 1 hour per day for 30 days on lipid levels, lipid peroxidation and lipase activity in rat brains. Lipid content and enzyme activity varied depending on brain area (Study ID ◆ 249).

Functional

Several studies investigated the effect of SO_2 exposure on respiratory reflex mechanisms. Rabbits were the species most frequently represented. However, other species examined included cats, ferrets, dogs and rats. These concur that bronchoconstrictive response is reflexive in nature. However, the mechanism of the reflex has not been conclusively determined (Barthelemy et al., 1988 – Study ID ▲ 197; Korpas and Widdicombe, 1983 – Study ID ◆ 153; Matsumoto et al., 1997 – Study ID ◆ 200; Wang et al., 1996 – Study ID ◆ 211; Davies et al., 1978b – Study ID ◆ 239; Davenport et al., 1984 – Study ID ◆ 244; Nadel et al., 1965 – Study ID ● 069; Mortola et al., 1985 – Study ID ● 141; Hanacek et al., 1991 – Study ID ● 161; Cho et al., 1968 – Study ID ● 167;

Citterio et al., 1985b – Study ID ▲ 194;
Citterio et al., 1985a – Study ID ● 195;
Davies et al., 1978a – Study ID ● 234;
Balchum et al., 1960 – Study ID ● 237).

Summary

Clinical and epidemiology
No human clinical or epidemiology studies were identified as fitting the criteria.

Non-clinical
Behavioural changes in rearing, social interactions, grooming, digging and chamber-crossing were reported in male and female mice exposed to 5, 12, and 30 ppm of near continuous exposure for 24 days. Male mice exposed to 5, 12, and 30 ppm prenatally exhibited changed aggressive behaviour in adulthood when subjected to an aggressive encounter with an unexposed mouse of the same age, body weight and isolation condition.
Changes in the lipid content of guinea pig and rat brains were reported for exposure to 10 ppm for 1 hour/day for 21 days and 30 days, respectively.
Several studies investigated the effect of SO_2 exposure on respiratory reflex mechanisms. These studies concur that the bronchoconstrictive response is reflexive, but the mechanism of the reflex has not been conclusively identified.

O. Olfactory System

Unlike for H_2S, there are no studies investigating the affect of SO_2 exposure on the sense of smell. Studies concerned with the effects of SO_2 on the nasal passages are described in the section on the respiratory system.

P. Reproductive System

Clinical studies

No clinical studies investigating this health outcome were located.

Non-clinical studies

Petruzzi et al. (1996) exposed adult male and female mice to 0, 5, 12, and 30 ppm SO_2 from 9 days before pregnancy to gestational day 12-14. There were no observed changes in reproductive performance or neurobehavioural development of the offspring (Study ID ▲ 214).
Singh (1982) investigated the teratogenicity of SO_2 exposure in mice. Pregnant mice were exposed to SO_2 concentrations of 0, 32, 65, 125, and 250 ppm from gestation days 7 through 17. On gestation day 18, the animals were sacrificed and the fetuses examined for teratological effects. No significant effect on the number of dead or reabsorbed fetuses and no significant teratological changes were observed. Several of the fetuses had hematomas at all levels of exposure and a significant decrease in the weight of pups exposed to 65 and 125 ppm was observed (Study ID ◆ 203)

Murray et al. (1979) investigated the embryotoxicity and teratogenicity of SO_2 in mice and rabbits exposed to 25 and 70 ppm, respectively. The mice were exposed on gestational days 6 through 15 and the rabbits were exposed on gestational days 6 through 18. SO_2 exposure had no effect on the dams of either species. The number of fetuses per litter and the number of reabsorptions were not affected by exposure to SO_2 in either species. However, mean fetal body weight was decreased in mice litters. (Study ID ◆ 140). Fiore et al. (1998) tested whether SO_2 exposure produces social or agonistic behavioural changes in adult male mice exposed to SO_2 prenatally. The mice were exposed to 0, 5, 12, or 30 ppm SO_2 on gestational days 1 through 14. At adulthood the exposed mice underwent an aggressive encounter with a non-exposed male of the same age and body weight. There was a significant enhancement in body sniffing behaviour and self-grooming duration in the exposed mice. Other non-social behaviours were increased, whereas behaviours such as tail rattling, freezing and defensive behaviours decreased. Offensive and attack behaviours were not modified (Study ID ◆ 217).

Epidemiology studies

Dolk et al. (2000) investigated whether populations living within a 7.5 km radius of cokeworks in Great Britain had a higher risk of adverse perinatal and infant outcomes. SO_2 is a major pollutant from cokeworks. Highest exposure was assumed to be at distances 2 km from the cokeworks. Outcomes were obtained from recorded birth and death data. No evidence of increased risk of low birth weight, infant mortality, neonatal mortality, postneonatal mortality, respiratory postneonatal mortality or postneonatal Sudden Infant Death Syndrome and proximity to cokeworks. However, this study had several shortcomings that limit the reliability and utility of these results, one being the lack of accurate SO_2 exposure data (Study ID ● 003).

Summary

Clinical

No human clinical studies investigating this health outcome were found.

Non-clinical

No significant teratological or embryotoxicological effects were reported in studies on mice exposed to up to 250 ppm during gestation.
No changes in reproductive performance or neurobehavioral development were reported in male and female mice exposed to up to 30 ppm during gestation. Some social or agonistic behavioural changes were reported during an aggressive encounter in adult male mice that had been exposed to up to 30 ppm during gestation.

Epidemiology

No moderate or high quality studies were identified for this health outcome.

Skin

Only one study mentioned effects of SO_2 exposure on skin (Study ID ▲ 159). This study was included in the **Signs and Symptoms** section of this report.

VI. Conclusions

The majority of the evidence from the scientific literature reviewed here refers to effects on the respiratory system. There is limited evidence of effects to other body systems, primarily from animal studies.

Evidence from Human Studies

The majority of the human studies investigated and reported respiratory effects. Both healthy subjects and those with respiratory illness (asthma or chronic obstructive pulmonary disease) were included in the studies.

The most common effects reported in healthy subjects were increased airway resistance and bronchoconstriction, decreased maximum expiratory flow, and decreased pulmonary function. Some subjects reported dryness and irritation of the throat, general respiratory discomfort, and unpleasant taste and odours. Effects reported in asthmatic subjects were similar, but also included increases in asthma symptoms, wheezing, chest tightness, and dyspnea. The evidence suggests that subjects with respiratory illness are more susceptible to respiratory health effects from SO_2 exposure.

Other factors contributing to SO_2-induced effects were examined in these studies. Exercise seems to exacerbate the response to SO_2 in both healthy and asthmatic subjects. Cold and/or dry air also exacerbates the asthmatic response. In addition, the method of exposure affects the response, with forced mouth breathing eliciting a greater response than nasal or oronasal breathing. Clinical studies covered a broad range of exposure durations. Therefore, the summaries of the findings are broken down by exposure time to facilitate comparison.

The weight of evidence for exposures up to 30 minutes suggests that healthy humans can experience exposures to SO_2 up to 10 ppm with only transitory effects on pulmonary function, even under challenging conditions involving hyperventilation, mouth-only exposure, and heavy exercise. Transitory effects may be observed at concentrations as low as 0.75 ppm.

For exposures up to 30 minutes, asthmatics appear to demonstrate pulmonary effects at lower thresholds (0.1 ppm), although even in this population subgroup the clinical effects are transient and may or may not require intermittent pharmacologic intervention.

The weight of evidence for single exposures up to 4 hours and repeated exposures between 3 days and 3 weeks suggests that transitory pulmonary effects might be expected for asthmatics at exposure concentrations between 0.5 and 1 ppm and for healthy humans between 0.75 and 25 ppm, with some evidence for a concentration-dependent response in healthy subjects.

No high quality epidemiology studies or case reports were identified.

Epidemiology studies were divided into two types based on calculation of exposure concentration. One set of studies calculated exposures as increases in ambient concentration above a baseline or average concentration. Another set of studies reported exposure as discrete concentrations, either as average concentrations or a concentration range.

A weight of evidence evaluation is difficult for the epidemiology studies as the majority of these studies were ranked low quality. For the moderate quality studies, there were an equal number of studies that found insignificant or no associations between ambient SO_2 concentration and health outcomes as there were that did report an association. These studies were subject to substantial limitations due to misclassification of both exposure and outcome. The majority of these studies are ecological in nature with outcomes determined on an individual level and exposure determined at a population level. The

exposure data collected was generally for ambient levels. Since humans spend a large portion of their time indoors and travel through various microclimates during various activities, ambient levels will likely not be a good measure of exposure at the individual level. Subsequently, the major weakness observed in these epidemiology studies is the potential for exposure misclassification as a result of the exposure assessment methods. Much of the exposure and outcome data used in these studies is retrospective and from public records, which increases the probability of misclassification due to inconsistent diagnosis of disease status and bias. Many confounding factors cannot be accounted for when using these types of data.
In addition, SO_2 is just one element in a mixture of pollutants found in "air pollution". It is difficult to isolate the effects of SO_2 from those of other single pollutants or combinations of pollutants. Because of these substantial limitations, the confidence in the results and conclusions from these epidemiology studies could not be judged to be higher than moderate and in most cases the confidence was judged to be low. Associations, when reported, were generally weak. Associations were reported for decreased pulmonary function, and hospital admissions for asthma and other respiratory diseases. Reported symptoms included throat irritation, chest discomfort, restricted activity, shortness of breath, cough, dyspnea, and lower baseline function. Weak associations were reported in epidemiology studies for various mortality causes. However, the body of epidemiological evidence for mortality contains much variability and few studies in which we can have confidence, mainly due to the limitations discussed above.

There is little reliable evidence in the peer-reviewed scientific literature meeting the terms of reference for this report of human health effects involving the eye, kidney and liver, or the cardiovascular, gastrointestinal, metabolic, immunological, reproductive, or nervous systems.

Evidence from animal studies
Much of the animal evidence for respiratory effects concentrates on the mechanisms of action of health effects from SO_2 exposure. The clinical significance of much of the animal evidence is unclear and was not discussed in the studies themselves. Studies on respiratory effects were well represented. Reported respiratory effects included increased bronchoconstriction and specific airway resistance and decreased ciliary activity. Non-clinical studies covered a broad range of exposure durations. Therefore, the summaries of the findings are broken down by exposure time to facilitate comparison.

The concentrations in respiratory studies of animals exposed for **up to 2 hours** ranged between 0.5 ppm and 1000 ppm. For concentrations up to 100 ppm, effects reported were predominantly very mild respiratory effects and changes at the cellular or ciliary level. Above 100 ppm, pulmonary effects were more in evidence, with indications of changes to the lung. There is evidence of increasing severity of effect with increasing concentration.

In studies with exposures **between 2 and 24 hours**, mild respiratory effects and delayed airway reactivity were reported with concentrations up to 40 ppm. Damage to the lungs was reported at concentrations of 800 ppm and 1225 ppm.

At exposures **between 1 and 7 days**, slight changes were observed in lung function and in response to virus challenges at concentrations between 0.1 ppm and 34.5 ppm. At the higher concentrations of 100 ppm and 600 ppm, changes to lung structure were reported.

Few respiratory studies investigated exposures **between 7 and 30 days**. One study reported changes in response to virus challenges with exposures up to 0.1 ppm. Other studies reported changes in lung biochemistry and some decrease in pulmonary function at concentrations between 10 and 600 ppm.

Only a few animal studies looked at the effect of SO_2 exposure on the liver or kidneys. However, there is some evidence of decreased levels of liver lipids and triglycerides and decreased enzyme activity in liver and kidney following SO_2 exposure.

There is some evidence that exposure to SO_2 can affect the metabolic system, in particular lipid metabolism, at exposure times of several days. This effect seems to differ depending on which organ of the body is investigated.
There is some evidence from animal studies that SO_2 exposure both as an adult and prenatally can affect behaviour in adult mice subjected to challenging conditions. There is also some evidence that exposure to SO_2 can affect the lipid content of the brain. The outcomes of both these studies are of unknown clinical significance and the number of studies is limited, although the quality of the studies suggests the results are reliable. It has been established in several species that bronchial restriction upon SO_2 exposure is a reflex reaction; however, the mechanism of this reflex has not been conclusively determined.

There is limited animal evidence for signs and symptoms, or effects on the eye, and reproductive, gastrointestinal, or cardiovascular systems found in the animal studies reviewed for this report.

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Key to Tables 1 through 9

Abbreviations for Tables
AC = children or adolescents with asthma or other chronic pulmonary disease
AA = adults with asthma or other chronic pulmonary disease
BAL = bronchoalveolar lavage
FEV_1 = forced expiratory volume in 1 second
FVC = forced vital capacity
HA = healthy adults
HC = healthy children or adolescents
$MEF_{50\%VC}$ = maximum expiratory flow from one half vital capacity
MMEF = maximum mid-expiratory flow
MEFR = maximum expiratory flow rate
MNPCE = polychromatic erythrocyte micronuclei
PEF = peak expiratory flow
ROI = reactive oxygen intermediate
SRaw = specific airway resistance
V_{max50} = maximum flow calculated at 50% vital capacity
V_{max75} = maximum flow calculated at 75% vital capacity

Confidence Index Ranking
High ▲
Moderate ◆
Low ●

Table 1A – Respiratory Effects Associated With Short-term Exposure to SO_2: Human Clinical Findings – 1 to 5 Minute Exposures

Concentration ppm *(mg/m³)*	Effects	Exposure Duration
0.1 ***(0.26)***	◆Bronchoconstriction at lower concentrations in dry air than humidified air (AA)[illegible]	3 min
0.5 ***(1.3)***	◆Increased SRaw (AA)[061], greater with oral than nasal exposure (AA)[074]; with cold dry air (AA)[121] ◆Dryness, irritation, or burning of the throat (to 5 ppm; HA)[019] ◆Chest tightness, wheezing, dyspnea (and 1 ppm; AA)[064] ◆Increased bronchoconstriction(AA)[126]	3min, 3x30 min/5/3 min 1 to 5 min 1, 3, 5 min 5 min
0.60 ***(1.6)***	◆Decreased respiratory function (AA)[114]	5 min
0.75 ***(2)***	◆Increase SRaw with hyperventilation[118]	5 min
<1 ***(<2.6)***	Chest tightness, wheezing, dyspnea, cough (AA)[091]	1,3, or 5 min
1 ***(2.6)***	◆Increased SRaw (AA)[062,064]	4 min/1,3,5 min
2 ***(5.2)***	◆Changes in SRaw (AA)[062]	4 min
10 ***(26)***	◆Bronchial obstruction, returned to control by 45-60 min. post-exposure (AA)[060]	3 min

Table 1B – Respiratory Effects Associated with Short-term Exposure to SO_2: Human Clinical Findings – 6 to 10 Minute Exposures

Concentration ppm *(mg/m³)*	Effects	Exposure Duration
0.2 ***(0.5)***	◆Increased respiratory frequency (AA)[071]	10 min
0.25 ***(0.6)***	◆Increased SRaw (AA)[118]	10 min
0.3 ***(0.8)***	◆Increased bronchoconstriction, returned to normal levels 30 min. post-exposure (and 0.6 ppm; AA)[097]	10 min
0.5 ***(1.3)***	▲Dose-dependent change in respiratory function (to 1 ppm; AA)[illegible] ◆Reduction in FEV$_1$, V_{max50}, V_{max75}, (AA)[055]	10 min 10 min
0.60 ***(1.6)***	◆Decreased respiratory function (AA)[104]	10 min
0.75 ***(2)***	◆Reduction in FEV$_1$, increased total respiratory resistance (AC)[103] ◆Increased SRaw[104]	10 min 10 min
1 ***(2.6)***	◆Reduction in FEV$_1$, V_{max50}, V_{max75} (AC)[038,102] ◆Slight reduction in FEV$_1$, V_{max50}, V_{max75} or increased bronchoconstriction after exercise (HC)[042]	30'r,10'e/10' 30'r,10'e
2 ***(5.3)***	◆Changes in SRaw (AA)[107]	10 min
2.5 ***(6.6)***	◆Decreased specific airway conductance greater with oral than nasal exposure (HA)[105]	10 min to 1 hr
5 ***(13)***	◆SRaw exhibited in all subjects[illegible]	10 min
15 ***(39)***	◆Increased pulmonary flow resistance; greater from oral than nasal exposure (HA)[054]	10 min

Table 1C – Respiratory Effects Associated with Short-term Exposure to SO_2: Human Clinical Findings – 11 to 30 Minute Exposures

Concentration ppm *(mg/m³)*	Effects	Exposure Duration
0.1 (0.3)	◆Slight reduction in FEV_1, $V_{max\ 50}$ (AC)[illegible]	15 min
0.5 *(1.3)*	▲Increased SRaw (AA)[109] ◆Dose-dependent effect on FEV_1, $V_{max\ 50}$, V_{max75} (to 1 ppm; AA)[099] ◆Increased SRaw -greater with oral than nasal exposure (AA)[074]	30 min 30 min 3x30 min
1 *(2.6)*	◆Reduction in FEV_1, $V_{max\ 50}$, V_{max75} (AC)[058], (AA)[110] ◆Slight reduction in FEV_1, $V_{max\ 50}$, V_{max75} or increased bronchoconstriction after exercise (HC)[042] ◆Decreased $MEF_{40\%VC}$ (HA)[070] ◆Functional impairment of alveolar macrophages (to 5.0 ppm)[308]	30'r,10'e/ 20 min 30'r,10'e 15 min 30 min
2 *(5.3)*	◆Difference in ventilatory parameters between forced oral and free-breathing exposures (HA)[266]	30 min
2.5 *(6.6)*	◆Decreased specific airway conductance greater with oral than nasal exposure (HA)[105] ◆Dose-dependent increase in ciliary beat frequency (to 12.5 ppm)[120,477]	10 min to 1 hr 30 min
4 *(10)*	◆Increased alveolar activity in BAL (and 8 ppm; HA)[083]	20 min
8 *(21)*	◆Increases in macrophages, lymphocytes, and mast cells in BAL (HA)[090]	20 min

Table 1D – Respiratory Effects Associated with Short-term Exposure to SO_2: Human Clinical Findings – 31 Minute to 4 Hour Exposures

Concentration ppm *(mg/m³)*	Effects	Exposure Duration
0.5 *(1.3)*	▲Increased SRaw (AA)[081] ◆Reduction in FEV_1, $V_{max\ 50}$, V_{max75} (AC)[099]	75 min 50 min
0.75 *(2)*	▲Increased SRaw (HA)[060] ◆Increased SRaw initially, decreased to pre-exposure levels after 1 hr of exposure (AA)[079]	2 hours 3 hours
1 *(2.6)*	▲Decreased spirometric function (HA)[096] ◆Increased SRaw (HA)[047], (AA)[078]	4 hr/d,3d/wkx3 2hr/1hr
2.5 *(6.6)*	◆Decreased specific airway conductance greater with oral than nasal exposure (HA)[105]	10 min to 1 hr
5 *(13)*	◆Decreased MMFR, increased bronchial clearance (HA)[047,056] ◆Decreased nasal mucous flow rate (HA)[048]	2.5 hr/3 hr 4 hr

Table 1E – Respiratory Effects Associated with Short-term Exposure to SO_2: Human Clinical Findings – >4 Hour Exposures

Concentration ppm *(mg/m³)*	Effects	Exposure Duration
1 *(2.6)*	▲Decreased spirometric function (HA)[096] ◆Decreased nasal mucous flow rate (HA)[063] ◆Discomfort proportional to SO_2 concentration (to 25 ppm; HA)[063]	4 hr/d,3d/wkx3 6 hr/d x 3d 6 hr/d x 3d

Table 2 – No Effects Associated With Short-term Exposure to SO_2: Human Clinical Findings

Concentration ppm *(mg/m^3)*	**Observations**	**Exposure Duration**
0.20 ***(0.52)***	◆ No significant effect on pulmonary function in asthmatics (AA)[067]	6 hours
0.40 ***(1.0)***	◆ No change in FEV_1 in healthy males with moderate exercise (HA)[057,089]	2 hours
0.50 ***(1.3)***	**▲ No significant effect in pulmonary function parameters for asthmatics (mouthpiece breathing; moderate exercise; from 0.25 ppm; AA)[075]** **▲ No effect on pulmonary function parameters (HA)[073]**	**1 hour (alternating rest with 10 min exercise)** 3 hours
0.60 ***(1.6)***	◆ No significant pulmonary effects for normal and atopic subjects with exercise (from 0.2 ppm; HA,AA)[109]	1 hour (incl. 3 – 10 min exercise periods)
0.75 ***(2.0)***	**▲ No effect on pulmonary function during or after exposure with exercise in healthy subjects (HA)[043]**	**4 hours (with 2 – 15 min exercise periods)**
0.80 ***(2.1)***	◆ No effect on pulmonary function for patients with COPD with exercise (from 0.4 ppm; AA)[101]	1 hour
1 ***(2.6)***	◆ No changes in pulmonary function for healthy subjects (from 0.25 ppm; HA)[106] ◆ No pulmonary function effects with exercise (HA)[122] ◆ No changes in pulmonary function or bronchial reactivity (HA)[040]	40 min (exercise) 2 hours (3 – 30 min exercise periods) 4 hr/d, 3d/wk for 3 wk
2 ***(5.2)***	◆ No changes in pulmonary function with free breathing, forced oral, and forced nasal (HA)[066] ◆No change in pulmonary function with exercise (HA)[047]	30 min 2 hours
3.6 ***(9.4)***	◆ No significant changes in pulmonary function parameters after exposure with normal breathing and hyperventilation (from 1.1 ppm; HA)[113]	30 min

Table 3A – "Positive" Respiratory Effects Associated With Short-term Exposure to SO_2: Animal Toxicology Studies – Up to 2 Hour Exposures

Concentration ppm (mg/m^3)	Effects	Exposure Duration
0.5 (*1.3*)	▲*Dose-dependent increases in lung resistance (and 5 ppm; Rabbits)*[197]	*45 min*
1 (*2.6*)	◆*Increased respiratory resistance; decreased compliance (Guinea pigs)*[229] ◆*Dose-dependent increase in bronchoconstriction (to 2.5 ppm; Guinea pigs)*[245,246] ◆*Decreased proportion of macrophages in white cells (to 5 ppm; Guinea pigs)*[245]	*1 hr* *10 min/1 hr* *10 min*
10 (*26*)	◆*Inhibition of ciliary movement (Rabbits)*[468]	*1 hr*
15 (*39*)	◆*Dose-dependent increased in ciliary activity (to 77 ppm; guinea pigs)*[213]	*2 to 6 min*
17 (*44*)	◆*Dose-dependent respiratory depression (to 298ppm; Mice)*[243]	*10 min*
50 (*131*)	▲*Reduction in pulmonary macrophage endocytosis(Hamsters)*[374] ◆*Reduced dynamic compliance (Dogs)*[189]	*Unreported* *15 min*
100 (*262*)	▲*Increase in minute volume (Chickens)*[183]	*60 min*
200 (*524*)	◆*Decreased breathing frequency, increased tidal volume (Rabbits)*[244]	*15 – 20 min*
500 (*1310*)	▲*Decreased SRaw(Chickens)*[183] ◆*Changes to bioelectric properties and increased nonelectrolyte permeability (Dogs)*[250]	*60 min* *75 min*
800 (*2096*)	◆*Reduction in minimal and maximal pulmonary surface tension (Rats)*[155]	*1 hr*
1000 (*2620*)	▲*Initial decrease then increase in SRaw, increased respiratory frequency, decreased minute volume (Chickens)*[183]	*60 min*

Table 3B – "Positive" Respiratory Effects Associated With Short-term Exposure to SO_2: Animal Toxicology Studies – 2 Hour to 1 Day Exposures

Concentration ppm (mg/m^3)	Effects	Exposure Duration
4 (*10*)	◆*Increased airway reactivity in asthmatic sheep 24 hr after exposure (Sheep)*[230]	*4 hr*
10 (*26*)	◆*Increased airway reactivity in asthmatic sheep 24 hr after exposure (Sheep)*[231] ◆*Lesions of olfactory and respiratory epithelium (Mice)*[191] ◆*Decrease in thickness of olfactory mucosa, severe rhinitis (Mice)*[191]	*4 hr* *4 to 72 hr* *4 to 72 hr*
20 (52)	◆*Delayed early clearance of upper respiratory tract (Rats)*[256]	*4 hr*
40 (*105*)	◆*Dose-dependent decrease in % SO_2 retention, respiratory rate, minute volume, increase in tidal volume (Rats)*[233]	*2 hr*
800 (*2096*)	◆*Gradient of decreasing damage in the tracheobronchial tree(Rats)*[104]	*8 hr*
1225 (*3210*)	◆*Pulmonary edema, greater reduction in surface tension (Rats)*[155]	*2 hr*

Table 3C – “Positive” Respiratory Effects Associated With Short-term Exposure to SO_2: Animal Toxicology Studies – 1 Day to 7 Day Exposures

Concentration ppm *(mg/m³)*	Effects	Exposure Duration
0.1 *(0.26)*	▲*Increased respiratory pause (Guinea pigs)*[239] ◆*Slight reduction in lung clearance (Rats)*[135] ◆*Increased antigen-specific antibodies in serum and bronchoalveolar fluid (Guinea pigs)*[133]	*5 hr/d, 5d* *70 hr* *8 hr/d, 5 d*
3.4 *(8.9)*	◆*Increased incidence of pneumonia after exposure to SO_2 (to 34.5 ppm; Mice)*[192]	*7 d*
6 *(16)*	◆Inhibition of virus growth (Mice)[238]	*7 d*
10 *(26)*	◆*Lesions of olfactory and respiratory epithelium (Mice)*[191] ◆*Decrease in thickness of olfactory mucosa, severe rhinitis (Mice)*[191]	*4 to 72 hr* *4 to 72 hr*
100 *(262)*	◆*Decreased glutathione concentration and inflammation (Rats)*[231]	*5hr/d, 7 to 28d*
600 *(1572)*	◆*Increased mucosal permeability (Rats)*[208]	*30 to 100 hr*

Table 3D – “Positive” Respiratory Effects Associated With Short-term Exposure to SO_2: Animal Toxicology Studies – Greater Than 7 Day Exposures

Concentration ppm *(mg/m³)*	Effects	Exposure Duration
0.03 *(0.08)*	◆ *More rapid and more severe inflammatory response to influenza infection (to 0.1 ppm; Mice)*[207]	*4 weeks*
10 *(26)*	◆*Increased concentrations of cholesterol, total lipids, gangliosides and decreased phospholipids (Guinea pigs)*[163]	*1 hr/d x 30d*
100 *(262)*	◆*Decreased glutathione concentration and inflammation (Rats)*[231]	*5hr/d, 7 to 28d*
150 *(393)*	◆*Increased lung resistance, decreased breathing frequency (Rabbits)*[236]	*12 x 3hr*
600 *(1573)*	◆*Increase in solid material recovered by bronchial lavage (Rats)*[230]	*3 hr/d for 9,18, or 30 d*

Table 4A Epidemiology: Mortality Endpoints Associated with Increases in Short-term Exposure to SO_2: Statistically Significant / Positive Findings

Exposure Increase ppb (μg/m³)	**Effect**	**Baseline Exposure ppb (μg/m³)**	**Study duration**	**Study Population**	**Reported Association**
4.0 ***(10)***	● Increase in daily death count for people >65 years old (lag 3d)[illegible]	1.9 – 22.9 *(5-60)*	2 years	N/A	2.4% Increase in daily death count
4.0 ***(10)***	● Weak increases in daily deaths associated with an increase in SO_2 concentration[illegible]	4.2 – 16.8 *(11-44)*	~6 years	N/A	RR: 1.0027 (95% CI: 1.0018 – 1.0073)
4.0 ***(10)***	● Slight association with respiratory mortality when SO_2 increased[illegible]	Mean: 6.4 ± 4.4 *(17±12)*	3 years	17 ± 5 respiratory deaths/day	RR: 1.015 (95% CI: 1.001 – 1.029)
5.7 ***(15)***	● Significant increase in stroke mortality with a 2d lag[illegible]	Mean: 12.1 ± 7.4 3.0 – 46.0 *(32±19)* *(7.9-121)*	3 years	15.3 stroke deaths/day	2.9% increase (95% CI: 0.8% - 5.0%)
Rouen: **7 – 14** ***(18-37)*** **Le Havre:** **4 – 13** ***(10-34)***	◆ Significant increases in respiratory mortality associated with increases in SO_2 concentrations[illegible]	Rouen: Summer: 9.1 *(24)* Winter: 13.5 *(35)* Le Havre: Summer:10.6 *(28)* Winter:15.1 *(40)*	5 years	Rouen: 21 883 deaths Le Havre: 13 885 deaths	Rouen: 8.2% increase (95% CI: 0.4%-16.6%) Le Havre: 3% increase (95% CI: 0.8% – 5%)
7.8 ***(20)***	● RR for total mortality >1 for neonates and people ≥65 yrs; RR for respiratory mortality >1 for people aged 2 -64[illegible]	Mean: 11.1 ± 7.0 2.4 – 46.0 *(29±18)* *(6-121)*	4 years	Total deaths: Postneonates: 1045 2-64 yrs: 67 597 ≥65: 100 316	N/A
17.43 ***(46)***	● Significant association between increased SO_2 and increases in ischemic stroke mortality[illegible]	21.8 ± 18.8 *(57±49)*	6 years	7.4 deaths/day from stroke	RR: 1.04 (95% CI: 1.01 – 1.08)
19 ***(50)***	● Significant association with increased total mortality, CV mortality, and respiratory mortality[illegible]	N/A	5 years	9 cities 260 000 – 9 million people/city	Total deaths RR: 1.036 (95% CI: 1.021 – 1.052)
19 ***(50)***	◆ Significant increases observed for respiratory and CV deaths[illegible]	Mean: 46.76 2.12 – 314.57 *(123)* *(5.6-824)* Max: 100.22 4.71 – 635.69 *(263)* *(12±1666)*	5 years	6.43 deaths/day	Total mortality RR: 1.06 (95% CI: 1.02 – 1.09) Respiratory mortality :1.05 (95% CI: 1.02 – 1.09) CV Mortality RR: 1.08 (95% CI: 1.03 – 1.12)
19 ***(50)***	◆ Significant increase in daily mortality in Western European cities[illegible]	Median: 5.0 – 28.2 *(13-74)* Mean winter: 11.4 – 125.9 *(30-330)*	5 – 14 years	12 cities	3% increase (95% CI: 2% - 4%)

Table 4A Epidemiology: Mortality Endpoints Associated with Increases in Short-term Exposure to SO_2: Statistically Significant / Positive Findings *(continued)*

Exposure Increase ppb (µg/m³)	Effect	Baseline Exposure ppb (µg/m³)	Study duration	Study Population	Reported Association
38 ***(100)***	◆ Association with total mortality, elder mortality (>70 yrs) and CV mortality for whole year and winter. Association with total mortality, elder mortality, CV and respiratory mortality in summer[147]	Mean 24-hour Winter: 17.6 0.8 – 61.1 *(46)* *(2-160)* Summer: 13.9 2.1 – 44.5 *(36)* *(5.5-117)*	6 years	Median total mortality: Winter: 48/d Summer: 43/d	All cause RR: 1.13 Elderly RR: 1.13 CV RR: 1.14
38 ***(100)***	◆ SO_2 increases associated with an increased risk of daily mortality[149]	Average: 19.6±11.4 *(51±30)* Range: 2.3 – 137.8 *(6-361)*	1826 days	Mean daily deaths: 37.2±8.0	RR: 1.12 (95% CI: 1.07 – 1.16)
38 ***(100)***	● Regression analysis estimates a significant association between increased SO_2 and increased mortality[illegible]	<76.3 ppb 19% of time *(< 200)* Max: 229 *(600)*	1167 winter days	17.3 deaths/d	RR:1.19
38 ***(100)***	● SO_2 increases associated with excess mortality[illegible]	Means: 40.8 – 83.6 *(107-219)*	8 years (winters)	1987 pop: 1 284 553	N/A
38 ***(100)***	● Associated with respiratory mortality (2d lag) and CV mortality (2 and 3d lag)[illegible]	Mean: 81.2 12.2 – 217.9 *(21)* *(32-571)*	365d	Mean daily deaths: Resp:9.6 CV:2.9	Resp RR (2d lag): 1.11 (95% CI: 1.02 0 1.22) CV RR (2d lag): 1.10 (95% CI: 1.02 – 1.20) (3d lag): 1.20 (95% CI: 1.11 – 1.30)
38 ***(100)***	● Significant increase in total mortality when using single pollutant model[illegible]	21 *(55)*	7 years	Population: 1 688 710	5% increase (95% CI: 3% - 7%)
38 ***(100)***	● Some significant associations depending on the combination of age, gender, and season used[illegible]	Annual mean: Station 1: 26.9±19.9 1.5 – 182.8 *(70±52)* *(3.9-479)* Station 2: 30.1±17.1 3.4 – 142.7 *(79±45)* *(8.9-374)*	6 years	Annual: Total deaths/d: 60.3±11.3 CV deaths/d: 23.7±6.5 Resp deaths/d: 5.8±3.1	N/A
100 ***(262)***	● Association with mortality in spring and winter[illegible]	Daily means: Spring: 16.8 *(44)* Summer: 15.7 *(41)* Fall: 17.8 *(47)* Winter: 25.4 *(67)*	15 years	Daily deaths: S: 54.4(75) S:51.0(78.3) F:52.6(66.5) W:59.3(97.4)	Spring RR: 1.19(95% CI: 1.06 – 1.33) Winter RR: 1.21 (95% CI: 1.09 – 1.35)

Table 4A Epidemiology: Mortality Endpoints Associated with Increases in Short-term Exposure to SO_2: Statistically Significant / Positive Findings *(continued)*

Exposure Increase ppb ($\mu g/m^3$)	Effect	Baseline Exposure (ppb)	Study duration	Study Population	Reported Association
355 ***(930)***	● Increase in mortality observed with increase in SO_2[illegible]	Median daily: Mean:75.2 3.8-1361 *(197)* *(10-3566)* Max:160 3.8-1892.7 *(419)* *(10-4960)*	10 years	Population: 107 000 Median deaths/d: 6	RR: 1.10 (p=0.01)
1 standard deviation (?)	● Increased SO_2 associated with increase in total deaths[illegible]	Mean: 2.8±1.7 0.3 – 15.4 *(7.3 ±4.5)* *(0.79-40)*	2 years	30-40 deaths/day	RR: 1.0027 (95% CI: 1.0018 – 1.0073)
"Doubling" concentrati on	◆ Risk of all cause mortality increase associated with increases in SO_2 concentration[138]	Mean: 39 *(102)* Max: 240 *(629)*	334 d	Population: 1 419 123	All cause increased risk: 11% (95% CI: 5% -16%)

Table 4B Epidemiology: Mortality Endpoints Associated with Increases in Short-term Exposure to SO_2: Insignificant / No Associations Found

Exposure Increase ppb ($\mu g/m^3$)	Effect	Baseline Exposure (ppb)	Study duration	Study Population	Reported Association
4 ***(10)***	● No significant association with total or CV mortality[illegible]	Mean: 22.6 7.2 – 52.8 *(59)* *(19-138)*	1 year	2.2 million	Total mortality RR: 1.007 (95% CI: 0.56 – 1.062) CV mortality RR: 1.028 (95% CI: 0.937 – 1.129)
4 ***(10)***	● No association with daily mortality[illegible]	Mean: 15.2±5.9 2.7 – 40.3 *(40±15)* *(6.3-106)*	2 years	Mean total daily mortality: 17.49±5.03	Total mortality RR: 1.007 (95% CI: 0.999 – 1.015) Total mortality (>70yrs)RR: 1.009 (95% CI: 1.00 – 1.21) CV mortality RR: 1.012(95% CI: 0.995 – 1.026)
4 ***(10)***	● For single city analysis of all 13 cities in study, there were no associations between SO_2 and mortality[illegible]	Daily mean range: 3.1 – 17.0 *(8.1-45)*	6 years	Mean total daily deaths: 2.5 – 60.9 Population ranges: 134 000 – 2.9 million	N/A

Table 4B Epidemiology: Mortality Endpoints Associated with Increases in Short-term Exposure to SO_2: Insignificant / No Associations Found *(continued)*

Exposure Increase ppb (µg/m³)	Effect	Baseline Exposure (ppb)	Study duration	Study Population	Reported Association
7.8 ***(20)***	● Total mortality RR<1 for 2-64 year olds; respiratory mortality RR<1 for postneonates and people ≥65 yrs[illegible]	Mean: 11.0±7.0 2.4 – 46.0 *(29±18)* *(6-121)*	4 years	Total deaths Postneonates: 1045 2-64 yrs: 67 597 ≥65: 100 316	N/A
12.9 ***(34)***	● No significant associations with total mortality[illegible]	Mean: 6.6±4.4 0.1 – 39.8 *(17±12)* *(0.26-104)*	14 years	N/A	RR: 1.08 (95% CI: 0.37 – 1.78)
7 – 17 ***(18-45)***	● No significant association between ambient SO_2 concentration increases and all-cause mortality in London[illegible]	24h average: 12.2±4.5 *(32±12)*	5 years	All cause deaths/d: 175.5±27.0	RR: 1.01 (95% CI: 1.00 – 1.03)
17.43 ***(46)***	● Insignificant association with hemorrhagic stroke mortality[illegible]	21.8±18.8 *(57±49)*	6 years	Stroke deaths/d: 7.4	N/A
19 ***(50)***	◆ Insignificant association with mortality for Central Eastern European cities[116]	Mean: 46.76 2.12 – 314.57 *(123)* *(5.6-824)* Max: 100.22 4.71 – 635.69 *(263)* *(12-1666)*	5 -14 years	12 cities	0.8% (95% CI: -0.1% - 2.4%)
38 ***(100)***	● No association with total daily mortality when gender, age, and cause of death are not separated out from entire population of North Bohemia[illegible]	Mean: 38.1±34.2 3.2 – 376.5 *(100±90)* *(8.4-987)*	12 years	Total all cause deaths: Men: 45 074 Women: 41 206	N/A
38 ***(100)***	◆ No association found for daily mortality, regardless of lag day[illegible]	Mean: 5.0 *(13)* Max: 53.0 *(139)*	6 years	713 000	Current day RR: 1.042(95% CI: 0.943 – 1.151) 1d lag RR: 1.048 (95% CI: 0.952 – 1.154) 2d lag RR: 1.016 (95% CI: 0.923 – 1.119)

Table 4B Epidemiology: Mortality Endpoints Associated with Increases in Short-term Exposure to SO_2: Insignificant / No Associations Found *(continued)*

Exposure Increase ppb ($\mu g/m^3$)	Effect	Baseline Exposure (ppb)	Study duration	Study Population	Reported Association
38 ***(100)***	● Insignificant association with total mortality (2 and 3d lag); insignificant association with respiratory mortality (3d lag)[illegible]	Mean: 81.2 12.2 – 217.9 *(213)* *(32-571)*	365d	Daily deaths: Total: 9.6 Resp: 2.1 CV: 2.9	Total mortality RR: 2d lag: 1.04 (95% CI 1.00 – 1.09) 3d lag: 1.04 (95% CI 0.99 – 1.08) Respiratory mortality: 3d lag: 1.00 (95% CI: 0.91 – 1.10)
38 ***(100)***	◆ Insignificant effect on respiratory mortality[143]	Mean 24-hour Winter: 17.6 0.8 – 61.1 *(46)* *(2-160)* Summer: 13.9 2.1 – 44.5 *(36)* *(5.5-117)*	6 years	Median total mortality: Winter: 48/d Summer: 43/d	N/A
38 ***(100)***	◆ Insignificant association with increased 24hr SO_2 concentrations and daily count of deaths[151]	Mean: 11.3 *(30)* Median: 8.8 *(23)* 5th centile: 2.7 *(7)* 99th centile: 47.7 *(125)*	5 years	Daily average deaths: 37	N/A
38 ***(100)***	● Insignificant associations depending on combination of gender, age, and season[illegible]	Annual mean: Station 1: 26.9±19.9 1.5 – 182.8 *(70±52)* *(3.9-479)* Station 2: 30.1±17.1 3.4 – 142.7 *(79±45)* *(8.9-118)*	6 years	Annual: Total deaths/d: 60.3±11.3 CV deaths/d: 23.7±6.5 Resp deaths/d: 5.8±3.1	N/A
100 ***(262)***	● Insignificant association with mortality in the summer and fall[illegible]	Daily means: Spring: 16.8 *(44)* Summer: 15.7 *(41)* Fall: 17.8 *(47)* Winter: 25.4 *(67)*	15 years	Daily deaths: S: 54.4(75) S:51.0(78.3) F:52.6(66.5) W:59.3(97.4)	Fall: RR = 1.14; 95% CI 1.00-1.29 Summer not reported
380 ***(1000)***	● No significant association between SO_2 and daily deaths[illegible]	Range of means: 69 – 159.9 *(181-419)*	14 winters	292 deaths/d	N/A
1 standard deviation (?)	● Insignificant association with respiratory deaths or CV deaths[illegible]	Mean: 2.8±1.7 0.3 – 15.4 *(7.3±4.5)* *(0.79-40)*	2 years	30-40 deaths/day	N/A

Table 4C Epidemiology: Mortality Endpoint Associated with Short-term Exposure to SO_2: Effects of Discrete SO_2 Concentrations or Comparisons of Concentrations – Positive / Statistically Significant Findings

Exposure level ppb ($\mu g/m^3$)	Effect	Study Duration	Study Population	Reported Statistical Association
Daily average: **5.4** ***(14)*** **0.7 – 10.5** ***(1.8-27.5)***	● Increased mortality risk associated with SO_2 exposure[illegible]	11 years	816 991 deaths Population: 10.8 million	1.4% increased risk ($p<0.01$)
6.5 ***(17)***	◆ Significant association with mortality during the cool season[illegible]	1096 days	N/A	Total mortality RR: 1.04 (95% CI: 1.02 – 1.07) Respiratory mortality RR: 1.04 (95% CI: 1.00 – 1.09) CV mortality RR: 1.07 (95% CI: 1.02 – 1.11)
8 – 47 ***(21-123)***	● Statistically significant increase in daily mortality for daily means in range[illegible]	4018 d	Median cases/day: 29	3% increase in daily mortality
17.25±10.73 ***(45±28)***	● SO_2 concentrations associated with excess deaths in poculation[illegible]	2192 d	243.2 deaths/day	20% excess deaths attributed to SO_2
Marseilles: 19.3 ***(51)*** **Lyons: 24.8** ***(65)***	● Significant association between SO_2 concentrations and respiratory mortality up to 10d after exposure[illegible]	N/A	2 cities	N/A
Zurich: 35.4±35.5 ***(93±93)*** **Basle: 26.5±25.3** ***(69±66)*** **Geneva: 40.2±32.7** ***(105±86)***	◆ Significant association with respiratory mortality in Zurich and Geneva; Significant association with CV mortality in Basle and Geneva; Significant association with total mortality and mortality in people >65 in Basle and Geneva[illegible]	5 years	Total mortality per day: 8.8 – 21.7	N/A
>40 ***(>105)***	● SO_2 concentration a significant predictor of respiratory mortality with 1d lag[illegible]	20 months	Population: 2.4 million Deaths/day: 22.1±4.9	N/A
>76.3 ***(>200)***	● Association between daily maximum SO_2 and daily mortality[illegible]	1167 winter days	Deaths/d: 17.3	Correlation coefficient: 0.141 ($p<0.001$)
>88.8 ***(>232)***	● Mean daily 1-hour maximum associated with daily respiratory mortality[illegible]	5 years	Deaths per day: 37	N/A
>190 ***(>498)***	● Excess mortality associated with SO_2 exposure[illegible]	4 years	• New York and New Jersey metropolitan area	2% excess mortality observed

Table 4C Epidemiology: Mortality Endpoint Associated with Short-term Exposure to SO_2: Effects of Discrete SO_2 Concentrations or Comparisons of Concentrations – Positive / Statistically Significant Findings *(continued)*

Exposure level ppb (µg/m^3)	Effect	Study Duration	Study Population	Reported Statistical Association
1 – 316 ***(2.6-828)***	◆ Significant positive association with SO_2 exposure and respiratory deaths[927]	10 years	~1.5 million	Same day RR: 1.12 (95% CI: 1.05 – 1.23) Previous day exposure RR: 1.16 (95% CI: 1.05 – 1.29)
200 (low) ***(524)*** **400 (high)** ***(1048)***	● Additional daily deaths associated with SO_2 exposure[illegible]	5 years	N/A	Additional 10 – 20 deaths expected

Table 4D Epidemiology: Mortality Endpoint Associated with Short-term Exposure to SO_2: Effects of Discrete SO_2 Concentrations or Comparisons of Concentrations – Statistically Insignificant Findings / No Associations

Exposure level ppb (µg/m^3)	Effect	Study Duration	Study Population	Reported Statistical Association
Mean: **6±4** ***(16±10)***	● No association between SO_2 concentration and respiratory mortality in children in Sao Paolo, Brazil[447]	1 year	Respiratory deaths/d: 3.04±2.11 Nonresp. Deaths/d: 5.41±2.44	N/A
50th centile average: 6.81 **5.0 – 9.1** ***(18)*** ***(13-24)***	● When all confounders are controlled for, SO_2 concentration does not have any effect on mortality in the Netherlands[illegible]	8 years	N/A	N/A
6.9 ***(18)***	◆ No significant association with any mortality measure during the warm season[464]	1096 d	N/A	Total mortality RR: 1.02 (95% CI: 0.99 – 1.04) Respiratory mortality RR: 1.02 (95% CI: 0.99 – 1.09) CV mortality RR: 1.01 (95% CI: 0.97 – 1.05)
<11.4 ***(<30)***	● Less mortality observed than expected at the reported concentrations[illegible]	4 years	New York and New Jersey metropolitan areas	1.5% less mortality than expected
Mean: **15±6** ***(39±16)***	● No association between changes in SO_2 concentration and mortality in Los Angeles County[443]	9 years	Total deaths/day: 152	N/A

Table 4D Epidemiology: Mortality Endpoint Associated with Short-term Exposure to SO_2: Effects of Discrete SO_2 Concentrations or Comparisons of Concentrations – Statistically Insignificant Findings / No Associations *(continued)*

Exposure level ppb (μg/m^3)	**Effect**	**Study Duration**	**Study Population**	**Reported Statistical Association**
Means: **W:16.3±18.1** ***(43±47)*** **Sp:7.7±7.2** ***(20±19)*** **S:4.5±1.9** ***(12±5)*** **F:7.8±6.4** ***(20±17)***	● No significant association with SO_2 concentration and total mortality or cause specific mortality for any season[394]	4 years	Total deaths: 19 062	Total mortality RR: 0.998 (95% CI: 0.96 – 0.99)
Low quintile: **<5 *(<13)*** **High quintile:** **>22 *(>58)***	● Insignificant positive association between neonatal respiratory mortality when comparing low to high quintiles[440]	2 years	Live births: 222 000 Neonate deaths: 1819 Postneonate deaths: 880	RR: 3.91 (95% CI: 0.90 – 16.9); p=0.062
Mean: **21 *(55)***	● No significant association between SO_2 and total mortality when TSP and SO_2 are considered simultaneously[403]	7 years	Population: 1 688 710 Deaths/day: Total: 22.1 Cancer: 10.5 CV: 0.89 Pneumonia: 1.44	N/A
Marseilles: **19.3 *(51)*** **Lyons: 24.8** ***(65)***	● No association with CV deaths[402]	N/A	2 cities	N/A
11.07 – 28.3 ***(29-74)***	● Inconsistent associations between SO_2 and CV mortalities – associations were both positive and negative[398]	4 cities: 2863 – 4747 days	Deaths/day: 13 - 27	N/A
0 – 40 ***(0-105)***	● No association between SO_2 and ability to predict respiratory mortality[412]	20 months	Population: 2.4 million Deaths/d: 22.1±4.9	N/A
Zurich: **35.4±35.5** ***(93±93)*** **Basle:** **26.5±25.3** ***(69±66)*** **Geneva:** **40.2±32.7** ***(105±86)***	◆ No association with respiratory mortality in Basle; No association with total, >65 mortality, or CV mortality in Zurich; Association between SO_2 and mortality is negative at high concentrations in Basle[403]	5 years	Total mortality/d: 8.8±21.7	N/A
Hourly max: **60** ***(157)***	◆ No association between SO_2 concentration and mortality[458]	2496 days of observation	N/A	N/A
>76.3 ***(>200)***	● Insignificant association with mortality in males ≤65 yrs old[394]	1167 winter days	Deaths/d: 17.3	N/A

Table 5A Epidemiology: Respiratory Health Effects Associated with Incremental Increases in Short-term Exposure to SO_2: Statistically Significant / Positive Findings

Exposure Increase ppb ***(µg/m³)***	*Effect*	*Baseline Exposure ppb* ***(µg/m³)***	**Study Duration***	**Study Population⁺**	**Reported Association**
2.5 (8-hr average) ***(6.6)***	● Increase in "bothersome" and "more severe" symptoms in asthmatics[illegible]	4.6 ± 3.0 *(12±7.9)*	3 months	N = 22	Bothersome OR: 1.23 (95% CI 1.06–1.41) More Severe OR: 1.36 (95% CI 1.08-1.71)
3.8 ***(9.9)***	● Association with hospital admissions for heart disease and COPD[illegible]	6.5 Range: 1.0-26.1 *(17)* *(Range: 2.6-68)*	1 year	Admissions: Resp.: 84-232/d CV: 54-177/d	≥65 yr OR 1.023 (95% CI 1.012-1.036) All ages OR 1.013 (95% CI 1.004-1.021)
4.0 (hourly) ***(11)***	● Increased "bothersome" asthma symptoms in children ages 10-16[illegible]	7.0 ± 4.0 *(18 ± 10)*			Same day OR: 1.31 (95% CI 1.10-1.55)
4.0 ***(11)***	◆ "Small" association with increased hospital respiratory admissions[423]	6.8 ± 4.7 *(18 ± 12)*	2 years	Avg. admissions/d: (Hong Kong/London) Asthma: 7.8/14.1 Resp.: 91.3/58.3 Cardiac: 98.7/121.1 IHD: 36.0/51.3	Not reported
4.4 ***(12)***	● Elevated risk for hospital admissions for asthma in children < 15 yrs old[illegible]	7.7 ± 3.3 *(20 ± 8.6)*	2 years	6436 asthma admissions	OR 1.11 (95% CI 1.06-1.17)
4.5 (6d moving avg.) ***(12)***	● Increased chronic lower respiratory disease emergency visits in elderly[illegible]	7.1 ± 4.0 *(19 ± 10)*	2 years	13 163 emerg. Visits (2300 respiratory)	18% Increase in visits (Range: 4.14%-31.85%)
5.3 ***(14)***	● Log-linear relation with incidence of acute childhood wheezy episodes in asthmatics[illegible]	8.4 ± 5.3 *(22 ± 14)*	~1 year	Cases: 1025 Controls: 4285	OR 1.12 (95% CI 1.05-1.19)
5.7 (warm) ***(15)*** **7.8 (cool)** ***(20)*** **6.9 (all-yr)** ***(18)***	● Increase in number of physician consults for respiratory disease[illegible]	7.8 ± 2.5 (warm) *(20 ± 6.6)* 8.4 ± 3.4 (cool) *(22 ± 8.9)*	~2 years	Avg. daily consults: Young: 73.9±50.2 Adult: 96.3±62.6 Elderly: 15.5±11.3	0-14 yr: Cool: 5.5% (2.4%-8.7%) All yr: 3.5% (1.4%-5.8%) 15-64 yr: Warm: 4.6% (1.5%-7.7%)
6.8 ***(18)***	● Increased daily GP consultations for lower respiratory disease in children (strongest association). Other tested associations were not statistically significant[illegible]	Mean daily (winter): 8.4 ± 3.4 *(22 ± 8.9)*	~2 years	Consults/d: (Asthma/other LRD) Young: 14.0/39.7 Adult: 17.8/73.8 Elderly: 3.6/41.4	5.8% (95% CI 1.6%-10.2%)
7.6 ***(20)***	● Increased emergency room visits for asthma[illegible]	14.5 ± 8.32 Range: 1.1-53.8 *(38 ± 22)* *(Range: 2.9-141)*	5 years	23 000 ER visits ~6000 admissions	Increase in visits: Same day = 1.80 1d lag = 2.90 2d lag = 2.64

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[*] Study duration: length of time of study; is not equivalent to length of exposure

[+] Study population: the definition of study population is different for each study and ranges from the populations of the city(ies) in which the study was conducted to hospital admissions for the health outcome of interest.

Table 5A Epidemiology: Respiratory Health Effects Associated with Incremental Increases in Short-term Exposure to SO_2: Statistically Significant / Positive Findings (continued)

Exposure Increase ppb ***(μg/m³)***	*Effect*	*Baseline Exposure ppb* ***(μg/m³)***	**Study Duration**[*]	**Study Population**[+]	**Reported Association**
9.5 ***(25)***	● Positive association with COPD emergencies in summer and winter[illegible]	------	~4 years	Daily mean emerg visits: Winter: 15.8 Summer: 8.3	COPD Increase: Winter: 6% Summer: 9%
9.5 ***(25)***	● SO_2 increases associated with additional COPD admissions per day at different average daily SO_2 concentrations (27,38, and 57 ppb)[illegible]	24-hr mean: 21.6 ± 8.6 Range: 6.5-61 *(57 ± 23)* *(Range: 17-160)* 1-hr mean: 54.1 ± 37.7 Range: 5.3-274.8 *(142 ± 99)* *(Range: 14-720)*	~1 year	Daily mean visits: 11.9±5.6	24 hr average=38ppb 0.70/day (p=0.01) 24hr average=57ppb 0.55/day (p=0.01) 24hr average=27ppb 0.70/day (p=0.04)
10 ***(26)***	◆ Increased lower respiratory symptoms in children from grades 2-5[426]	Effects only seen once ambient conc. >22ppb *(>57)*	1 year	1844 Subjects	OR 1.28 (95% CI 1.13-1.46)
15 ***(39)***	◆ Increased lower respiratory symptoms in children with high BHR and serum IgE[440]	24hr mean: 3.2-8.6 *(8.4-23)*	3 winters (3 months each)	459 Subjects	Lag0 OR: 1.49 (95%CI 1.17-1.77) Lag1 OR: 1.28 (95%CI 1.00-1.64) Lag2 OR: 1.58 (95%CI 1.08-1.77) 5-day mean OR: 2.49 (95%CI 1.54-4.04)
19 ***(50)***	◆ Increased risk of asthma attack incidence in children ages 7-10[448]	8.3 ± 5.2 Range: 1.7-32.0 *(22 ± 13)* *(Range: 4.5-84)*	25 weeks	Mild: 43 subjects Moderate: 47 subjects	Same day OR: 2.86 (95% CI 1.31-6.27) 1d Lag OR: 2.45 (95% CI 1.01-5.92)
19 ***(50)***	◆ Association between daily mean and 1-hr SO_2 increase and hospital admission for COPD during warm season[369]	17.9 – 31.3 *(47-82)*	~5 – 12 years exposure	Admissions/d: 1.1 - 11	1-hr OR: 1.02 (95% CI 1.00-1.04) Daily OR: 1.05 (95% CI 1.01-1.10)
19 ***(50)***	● Increased hospital admissions for respiratory disease in elderly patients (>65yrs) in New Haven and Tacoma[illegible]	New Haven mean: 29.8 *(78)* Tacoma mean: 16.8 *(44)*	~2 years	Mean admissions/d: New Haven: 8.8 Tacoma: 4.2	New Haven RR 1.03 (95% CI 1-1.13) Tacoma RR 1.06 (95% CI 1.01-1.12)

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[+] Study population: the definition of study population is different for each study and ranges from the populations of the city(ies) in which the study was conducted to hospital admissions for the health outcome of interest.

Table 5A Epidemiology: Respiratory Health Effects Associated with Incremental Increases in Short-term Exposure to SO_2: Statistically Significant / Positive Findings (continued)

Exposure Increase ppb (*µg/m³*)	*Effect*	*Baseline Exposure ppb* **(*µg/m³*)**	**Study Duration***	**Study Population⁺**	**Reported Association**
19 *(50)*	● Association with wheeze (5 and 6d lag), cough (6d lag) and shortness of breath (1wk lag) in asthmatic adults (mean age 46 yr)[illegible]	8.3 ± 5.2 Range 1.7-32.0 *(22 ± 13)* *(Range: 4.5–8.4)*	6 months	40 subjects	Wheeze: 5d lag OR: 1.35 (95% CI 1.01-1.81) 6d lag OR: 1.39 (95% CI 1.04-1.87) Cough: 6d lag OR 1.34 (95% CI 1.00-1.79) Shortness of Breath: 1 wk lag OR: 1.56 (95% CI 1.06-1.72)
19 *(50)*	● Increased emergency room visits for asthma in patients ages 5-34 yrs in 3 cities[illegible]	Daily mean 13.7 ± 9.6 *(36±25)* 15.0 ± 9.7 *(39±25)* 4.2 ± 3.2 *(11±8.4)*	~5 years	4416 subjects	Visit increase: 12% (95% CI 1%-23%)
25 (in 5d mean) *(66)*	●Significant decrease in evening PEF -1.67 (-2.76 to -0.58) L/min, increase in phlegm and runny nose in asthmatic children[illegible]	Mean: 27.1 *(71)* Max : 146.2 *(383)*	7 months	89 subjects	Phlegm OR : 1.16 (95% CI 1.11-1.21) Runny Nose OR: 1.07 (95% CI 1.04-1.10)
38 *(100)*	◆ Increase in asthma (5% significance) and acute respiratory disease (1% significance) admissions in winter[140]	Winter mean daily level: 13.0 ± 0.39 *(34 ± 1.0)*	~2 years	N/A	Asthma admission increase: 4 (95% CI 0-7) Resp. disease admission increase: 15.5 (95% CI 6-25)
IQR (?)	● Significant % decrease in morning PEFR in children (age 4-9 years) with asthma[illegible]	Average daily 53 Range 5-75 *(139)* *(Range 13-197)*	1 year	Mean # of COPD Emergency Visits: 11.9±5.6	OR: 1.48 per IQR (2 d lag)

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* Study duration: length of time of study; is not equivalent to length of exposure

⁺ Study population: the definition of study population is different for each study and ranges from the populations of the city(ies) in which the study was conducted to hospital admissions for the health outcome of interest.

Table 5B Epidemiology: Respiratory Health Effects Associated with Incremental Increases in Short-term Exposure to SO_2: Insignificant / No Associations Found

Exposure Increase ppb ***(µg/m³)***	*Effect*	**Baseline Exposure ppb** ***(µg/m³)***	**Study Duration***	**Study Population⁺**	**Reported Association**
1.7 ***(4.5)***	● No association with morning or evening PEFR in COPD patients. Insignificant associations with eye irritation[illegible]	0-15 *(0-39)*	3 months	40 Subjects	Morning PEFR: 0.05% (95% CI -0.24 to 0.36%) Evening PEFR: -0.06% (95% CI -0.23 to 0.11%) Eye irritation RR: 1.17 (95% CI 0.99-1.38)
2.5 (8-hr) ***(6.6)***	● No significant association with asthma symptoms considered "bothersome" or "more severe" on a 1d lag in children ages 10-16[illegible]	4.6 ± 3.0 Range: 1-20 *(12 ± 7.8)* *(Range: 2.6-52)*	3 months	22 Subjects	Bothersome OR: 1.11 (95% CI 0.97-1.28) More Severe OR: 0.91 (0.51 – 1.60)
3.5 ***(9.1)***	● No association with hospital admissions and pneumonia in the elderly after a 2d lag[illegible]	4.8-6.6 *(13-17)*	~5 years	Respiratory mortality/d: Minnesota: 10.55 Birmingham: 8.26	Increase in admissions 1.6% (95% CI -0.1% to 3.3%)
3.8 ***(10)***	● No definitive association between SO_2 increases and respiratory, cardiac, cerebrovascular, and peripheral vascular diseases. Statistical significances were not reported[illegible]	5.4 *(14)*	~14 years	Total admissions: 449 278	2.8% excess daily hospital admissions ("completely explained by other variables")
3.8 ***(10)***	● No significant association with hospital emergency visits for asthma[illegible]	24 hr = 10.2 *(27)* 1 hr = 21.5 *(56)*	~1 year	734 asthma cases	All CI included 1
3.8 ***(10)***	◆ No association evident for exacerbation of symptoms in patients with COPD, regardless of lag or season[illegible]	Summer mean: 2.7 ± 1.9 Range: 0.8-10 *(7.1 ± 5.0)* *(Range: 2-26)* Winter mean: 7.3 ± 4.6 Range: 1.1-30.9 *(19-12)* *(Range: 2.9-81)*	14 months	39 Subjects	OR did not deviate significantly from 1

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[*] Study duration: length of time of study; is not equivalent to length of exposure

[⁺] Study population: the definition of study population is different for each study and ranges from the populations of the city(ies) in which the study was conducted to hospital admissions for the health outcome of interest.

Table 5B Epidemiology:. Respiratory Health Effects Associated with Incremental Increases in Short-term Exposure to SO_2: Insignificant / No Associations Found (continued)

Exposure Increase ppb *(μg/m³)*	*Effect*	**Baseline Exposure ppb *(μg/m³)***	**Study Duration***	**Study Population**⁺	**Reported Association**
3.8 ***(10)***	◆ No association between asthma exacerbation and mean 24-hour increases, regardless of lag or season[402]	Summer mean: 2.7 ± 1.9 Range: 0.8-10 *(7.1 ± 5.0)* *(Range: 2-26)* Winter mean: 7.3 ± 4.6 Range: 1.1-30.9 *(19-12)* *(Range: 2.9-81)*	1 year	60 Subjects	All CI included OR=1
4.0 ***(11)***	◆ No significant association with emergency visits for COPD for patients >14 yr, regardless of lag or season[431]	1-hr Mean: 21.5 Range: 3.4-60.1 *(56)* *(Range: 9-157)* 24-hr mean: 10.2 Range: 2.0-26.1 *(27)* *(Range 5.2-68)*	1 year	1298 COPD admissions	All RR were below 1 and were not statistically significant
4.0 ***(11)***	◆ No significant association with daily hospital asthma admissions in Hong Kong or London, or respiratory disease admissions in London[423]	Hong Kong daily mean: 6.8 ± 4.7 *(18 ± 12)* London daily mean: 9.0 ± 4.7 *(24 ± 12)*	2 years	Avg. admissions/d: (Hong Kong/London) Asthma: 7.8/14.1 Resp.: 91.3/58.3 Cardiac: 98.7/121.1 IHD: 36.0/51.3	No statistically significant associations
4.0 ***(11)***	● No significant associations wild mild asthma exacerbation (1d lag), and no association with moderate exacerbation for same day or 1d lag in children ages 10-16[411]	8-hr average: 7.0± 4.0 *(18 ± 10)*	3 months	22 subjects	Mild symptoms (1d lag) OR: 1.11 (95% CI 0.91-1.38) Moderate symptoms Same day OR: 1.37 (95% CI 0.87-2.18) 1d lag OR: 0.76 (95% CI 0.35-1.64)
5.7 (warm) ***(15)*** **7.8 (cool)** ***(20)*** **6.9 (all-yr)** ***(18)***	● No statistical significance for any increased physician consults for respiratory disease in elderly, or young (0-14 yr) in warm season, or adults (15-64) in cool season or all-yr average[410]	7.8 ±2.5 (warm) *(20 ± 6)* 8.4 ± 3.4 (cool) *(22 ± 9)*	~ 2 years	Avg. daily consults: Young: 73.9±50.2 Adult: 96.3±62.6 Elderly: 15.5±11.3	3.5% change (95%CI 1.4%-5.8%)

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* Study duration: length of time of study; is not equivalent to length of exposure

⁺ Study population: the definition of study population is different for each study and ranges from the populations of the city(ies) in which the study was conducted to hospital admissions for the health outcome of interest.

Table 5B Epidemiology: Respiratory Health Effects Associated with Incremental Increases in Short-term Exposure to SO_2: Insignificant / No Associations Found (continued)

Exposure Increase ppb *(µg/m³)*	*Effect*	Baseline Exposure ppb *(µg/m³)*	Study Duration*	Study Population⁺	Reported Association
6.8 ***(18)***	● No association with % change in daily GP visits for asthma or lower respiratory disease in adults and elderly[460]	Mean daily (summer): 7.8 ± 2.5 *(20 ± 6)* (winter): 8.4 ± 3.4 *(22 ± 9)*	~2 years	Consults/d: (Asthma/other LRD) Young: 14.0/39.7 Adult: 17.8/73.8 Elderly: 3.6/41.4	Associations for adults and elderly independent of SO_2 concentrations
9.5 ***(25)***	● No statistically significant association with hospital admissions or emergency visits for asthma in people > 14 yrs old[453]	Daily means: Summer: 40.8 *(107)* Winter: 52.0 *(136)*	460 d	N/A	No associations with SO_2 exposure
10 ***(26)***	● No association with asthma hospital admissions in people <65 yrs old[452]	Mean daily: 8.0 *(21)*	~7 years	Admissions: 7837 asthma 6437 appendicitis	No associations with SO_2 identified
10 ***(26)***	● No significant association with increased emergency admissions for cardiopulmonary ill health for a long study period and a short study period[illegible]	Mean daily: Long period: 14.5 ± 9.0 *(38 ± 24)* Short: 8.3 ± 5.6 *(22 ± 15)*	Short: ~3 years Long: ~14 years	All Cause Deaths/d: Short: 14.3 Long: 15.1±4.4	Short period: ≥65: CV: 4.9% (95% CI -1.0 to 11.0%) Resp.: -2.5% (95% CI -11.0 to 6.9%) ≥65: CV: -3.7% (95% CI -12.4 to 5.9%) Resp.: 0.0% (95% CI -8.3 to 9.1%)
10 ***(26)***	● No significant association with asthma symptoms in children ages 5-12, regardless of lag period[illegible]	1-21 *(2.6-55)*	~2 years	133 Subjects	Same day OR: 1.07 (95% CI 0.90-1.27) 1d lag OR: 1.07 (95% CI 0.90-1.28) 2d lag OR: 1.00 (95% CI 0.83-1.20)
10 ***(26)***	◆ Cough incidence not significantly associated with increased SO_2 for all concentrations, no significant association with upper respiratory symptoms, no significant association with lower respiratory symptoms at ambient concentrations<22 ppb[426]	Max. 24-hr average: 82 *(214)* (cough incidence) Other effects investigated below 22 *(58)*	1 year	1844 Subjects	Cough incidence OR: 1.0 (95% CI 0.90-1.10)
19 ***(50)***	◆ No significant association with all ages COPD symptom admissions[369]	1hr means: Amsterdam: 19 *(50)* Barcelona: 23 *(60)* Paris: 18 *(47)* Rotterdam: 31 *(81)*	~5 – 12 years	COPD Admissions: 1.1 - 11	OR 1.02 (95% CI 0.98-1.06)

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⁺ Study population: the definition of study population is different for each study and ranges from the populations of the city(ies) in which the study was conducted to hospital admissions for the health outcome of interest.

Table 5B Epidemiology: Respiratory Health Effects Associated with Incremental Increases in Short-term Exposure to SO_2: Insignificant / No Associations Found (continued)

Exposure Increase ppb ***(µg/m³)***	*Effect*	**Baseline Exposure ppb** ***(µg/m³)***	**Study Duration**[*]	**Study Population**[+]	**Reported Association**
.19 ***(50)***	● Nocturnal cough (3 & 4d lag) not associated for entire study group. For asthmatics there was no association with wheeze and 1 week lagged SO_2	Mean: 8.3 ± 3.2 Range: 1.7-32.0 *(Mean: 22 ± 8.4)* *(Range: 4.4-84*	6 months	40 Subjects	Nocturnal cough OR 3d: 1.60 (95% CI 0.98-2.62) 4d: 1.71 (95% CI 0.86-3.40) Wheeze (asthmatics): 1.64 (95% CI 0.91-2.94)
50 ***(131)***	● No statistically significant increase in hospital visits for asthma symptoms in children <16 yrs	Mean: 70 Range: 10-490 *(183)* *(Range: 26-1284)*	6 months	Population of children: ~450 000	No significant % increase identified
51 ***(134)***	● No statistically significant association with decreases in PEF in children (7-15yr) with asthma	Range of means: 27.1-90 *(71-236)*	2 years	257 Subjects	No statistically significant associations found
IQR (?)	● No effect on evening PEFR for children with asthma	Average daily 53 Range: 5-75 *(139)* *(Range: 13-197)*	1 year	COPD Emergency visits/d: 11.9±5.6	No association with evening PEFR

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Table 5C Epidemiology: Respiratory Health Effects Associated with Short-term Exposure to SO_2: Effects of Discrete SO_2 Concentrations or Comparisons of Concentrations – Positive/Statistically Significant Findings

Exposure Level ppb *(µg/m³)*	Effect	Study Duration*	Study Population+	Reported Statistical Association
Summer: 1.65-3.97 ***(4.3-10)*** **Winter: 2.21-5.14** ***(5.8-14)***	◆ Total respiratory admissions with and without asthma are correlated with summer SO_2 levels lagged 48 hours[illegible]	9 years Jan/Feb & Jul/Aug	Total admissions: Summer: 1 282 064 Winter: 1 223 456	Not reported
Low: 3.04 *(8.0)* **High: 4.94 *(13)***	● Significant association for asthma exacerbations with colds and levels of SO_2 which were significantly higher for a 3 day mean during warmer months[illegible]	~19 months	57 Subjects	p<0.1
Daily mean: 5.4 ± 3.0 **Range 1.5-16.9** ***(14 ± 8)*** ***(Range: 4 – 44)***	● Variation in clinic visits for lower respiratory tract illness is significantly associated with variations in SO_2 concentration[illegible]	1 year	Population at risk: 19 000 – 278 000	Not reported
Daily mean: 5.5 ± 5.7 ***(14 ± 15)***	●For children aged 7 – 11 yrs., significant association between daily prevalence of cough and same-day SO_2 concentration. Significant association between previous-day SO_2 concentrations and lower respiratory symptoms[illegible]	3 consecutive winters	Winter 1: 308 subjects Winter 2: 381 subjects Winter 3: 390 subjects	Cough OR: 1.10 LRD OR: 1.19
Average daily means: 5.0 – 9.5 ***(13 ± 25)***	● Hospital admissions for asthma associated with daily SO_2 levels in age groups 15-64 yrs and >64 yrs. Significant associations also seen for the control disease and pollutant levels[illegible]	2 years	2421 admissions	15-64: (p=0.046) >64: (p=0.012)
Daily mean: 7.3 ± 4.8 **Range: 0.08-36.1** ***(19 ± 13)*** ***(Range: 0.21-95)***	● Frequency of admissions for asthma (all age groups) were significantly correlated with daily levels of SO_2[illegible]	3 years	4209 admissions for asthma	7% more admissions during higher pollution.
Mean 1983 – 1987: 9.1 ± 3.1 ***(24 ± 8)***	● Childhood asthma hospital admissions correlated with monthly and quarterly mean SO_2 concentrations[illegible]	~4 years	Population: 100 000 Asthma visits Emergency: 921 Clinic: 2183	Month: r=0.334 (p=0.01) Quarter: r=0.473 (p=0.07)
> 15 ***(39)*** **Range of 24hr means: 6.5 to 6.8** ***(17-18)***	◆ Increased SO_2 concentrations corresponded to decreased peak flow levels at concentrations above 15 ppb	8 months	27 nonallergic asthmatics aged 18-60 years from two cities	N/A
Peak hourly: **Low: 20 – 40 *(52-105)*** **High: 110 – 150 *(288-393)***	◆ Significant increases in eye and throat irritation, chest discomfort, shortness of breath, restricted activity, and medical visits during elevated pollution episodes compared to low pollution[illegible]	1 summer	1121 Subjects	Not reported
Peak concentrations: **24-hr: 40 *(105)*** **1-hr: 56 *(147)***	◆ Winter air pollution events (elevated pollutant concentrations) associated with prevalence of wheeze and bronchodilator use, and decreased PEF in children with chronic respiratory symptoms[illegible]	1 winter	73 Subjects	Not reported

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Table 5C Epidemiology: Respiratory Health Effects Associated with Short-term Exposure to SO_2: Effects of Discrete SO_2 Concentrations or Comparisons of Concentrations – Positive/Statistically Significant Findings (continued)

Exposure Level ppb ***(µg/m³)***	**Effect**	**Study Duration***	**Study Population⁺**	**Reported Statistical Association**
Low: 40 ***(105)*** **High: >250** ***(>655)***	◆ Patients age 55+ with grade 3 and 4 bronchitis, exposure to high SO_2 compared to low resulted in 50% greater person days of acute respiratory illness[illegible]	10 months	561 Subjects	Not reported
Means **At least >57** ***(149)*** **Up to 171** ***(448)***	● During first 13 weeks of study, correlation between SO_2 concentrations and children's respiratory morbidity. No clear exposure levels reported[illegible]	4 months	N/A	Not reported
0 – 190 ***(0-498)***	◆ Increased prevalence of upper airway symptoms and nasal catarrh in exposed workers (n=136) versus controls. FEF_{25}, FEF_{50}, and FVC lower in subjects compared to controls[609]	"Years"	223 Subjects	Not reported
Average daily: 470 ***(1232)***	◆ Smelter workers (n=36) had higher prevalence of dyspnea and lower baseline lung function compared to unexposed controls[118]	2 weeks	67 Subjects	Not reported

Table 5D Epidemiology: Respiratory Health Effects Associated with Short-term Exposure to SO_2: Effects of Discrete SO_2 Concentrations or Comparisons of Concentrations– Statistically Insignificant Findings/No Associations

Exposure Level ppb ***(µg/m³)***	**Effect**	**Study Duration***	**Study Population⁺**	**Reported Statistical Association**
Mean: **Summer: 1.3** ***(3.4)*** **Winter: 2.6** ***(6.8)*** **24-hr range: 0.1-30** ***(0.26-78)*** **Maximal 1-hr: 50.0** ***(131)***	● No association between frequency of registration of patients with acute asthma in an emergency department and concentration of SO_2[illegible]	~40 months	Population: 120 000 N=4127 asthma visits	No association
Low: 3.04 ***(8.0)*** **High: 4.94** ***(13)***	● No association evident between asthma exacerbations with colds and daily levels of SO_2 in the colder months of the year, or with SO_2 concentrations 1-day before an event[illegible]	18 months	57 Subjects	No association
Daily mean: 5.5 ± 5.7 ***(14 ± 15)***	● For children ages 7 – 11, no significant association between acute respiratory symptoms and SO_2 concentrations. SO_2 concentration not associated with pulmonary function[illegible]	3 consecutive winters	Winter 1: 308 subjects Winter 2: 381 subjects Winter 3: 390 subjects	No association
Average daily means: 5.0 – 9.5 ***(13-25)***	● For 0-14 yr, no significant association between SO_2 level and hospital admissions for asthma[illegible]	~2 years	2421 total admissions	No association

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⁺ Study population: the definition of study population is different for each study and ranges from the populations of the city(ies) in which the study was conducted to hospital admissions for the health outcome of interest.

Table 5D Epidemiology: Respiratory Health Effects Associated with Short-term Exposure to SO_2: Effects of Discrete SO_2 Concentrations or Comparisons of Concentrations– Statistically Insignificant Findings/No Associations (continued)

Exposure Level (ppb)	Effect	Study Duration*	Study Population+	Reported Statistical Association
Mean: 7.0 ***(18)***	● No association with asthma hospitalizations and SO_2 for boys aged 6 – 12 (all lag periods). No association for girls aged 6 – 12 for lag periods under 6d.[illegible]	~12 years	7319 asthma hospitalizations	No association
Mean 1983 – 1987: 9.1 ± 3.1 *(24 ± 8)*	● No correlation between daily levels of SO_2 and asthma attack rates in children < 10 yrs.[illegible]	~4 years	Population: 100 000 Asthma visits Emergency: 921 Clinic: 2183	No association
Daily levels: Low: 0 ***(0)***** High: >10.9 *(29)***	● No correlation between SO_2 daily maximums and prevalence of wheezing or any other symptoms in asthmatic schoolchildren[illegible]	1 year	99 Subjects	No association
Weekly mean: 3.8 – 24.5 *(10-65)*	● Correlation between SO_2 concentrations and ER visits for asthma in children investigated. No statistical significance calculated[illegible]	1 year	1076 Subjects	No association
Concentration range: 11 – 27 *(29-71)*	● No significant association between SO_2 concentrations and incidence of respiratory symptoms in children aged 2 - 5[illegible]			No association
Annual Concentrations: Low: <15.3 *(40)* High: 30.5 ***(80)***	◆ Exposure to higher concentrations of SO_2 not the cause of acute respiratory illness, when areas of low concentration exposure were compared to high[618]	3 years	4 Regions 240-280 families/region	No association
Median: 16 Range: 11-55	● A consistent, significant association between SO_2 exposure and peak expiratory flow was not observed[illegible]	4 months	60 asthmatic children	No association
Daily levels: 0 – 38 *(0-100)*	◆ In children age 7 – 12, no association between prevalence of acute respiratory symptoms and concentrations of air pollutants during winter pollution episodes[618]	14 days	112 Subjects	No association
Range of daily averages: 0 – 38 *(0-100)*	● No significant association with air concentration and total number of medical contacts for respiratory illness in children ages 0 – 15[illegible]	4 months	65 297 children 5307 contacts (3974 respiratory contacts)	p=0.68
Nonpolluted area: 13 – 27 *(34-71)* Polluted area: 19 – 40 *(50-105)*	● No correlation between SO_2 levels and total respiratory disease morbidity rates for all ages for pollution events in both the low and high polluted areas[illegible]	9 weeks/yr 3 years	Population base: 187 000	No association
Daily range: Rural: 1.3-24.8 *(3.4-65)* Urban: 1.0 - 43.5 *(2.6-114)*	● No clear associations with PEF, respiratory symptoms, or bronchodilator use in children aged 6 – 12[illegible]	2 months	Total Subjects 2010 14 studies – Avg pop/study: Urban: 50-91 Suburban: 60-84	No association

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Table 5D Epidemiology: Respiratory Health Effects Associated with Short-term Exposure to SO_2: Effects of Discrete SO_2 Concentrations or Comparisons of Concentrations– Statistically Insignificant Findings/No Associations (continued)

Exposure Level ppb (μg/m³)	Effect	Study Duration*	Study Population+	Reported Statistical Association
5-day average Mean: 73.4 ± 40 *(192 ± 014)* **Range: 14.7 – 275.5** *(Range: 39-722)*	● Changes in SO_2 concentrations are not associated with FEV_1 and MEFR in patients with COPD[illegible]	1 year	18 Subjects	No association
Mean hourly exposure: 150 – 660 *(393-1730)*	● Temporary work in a greenhouse at the reported high concentrations did not affect lung function[illegible]	6.6 years	42 Subjects	No association
Exposures up to 610.6 *(1600)*	● No significant correlation between PEF on arrival at work each day and pollution index[illegible]	Up to 2 years	4 Subjects	No association
Exposures of 0 – 1500 *(0-3931)*	◆ No significant correlation between SO_2 and asthma emergency room visits, despite comparisons of results on a daily, weekly, and monthly basis[923]	1 year	854 Emergency visits	No association
Plant 1: 1030 *(2700)* **Plant 2: 200 – 1800** *(524-4720)*	● Causative role of exposure and irritative effects in cement plant workers undetermined[illegible]	Plant 1: 5-6 months Plant 2: 6-8 months	N/A	No association
Up to 2135.0 *(5595)*	● No significant associations with FEV_1, FVC, and MMEF in 4 subjects[illegible]	5 years	4 Subjects	No association
Range of exposures: 5- minute block mean: 0 – 3319 *(0-8698)*	◆ No evidence of any positive relation between peak SO_2 concentrations and hospital presentations or admissions for asthma, wheeze, or shortness of breath[1007]	3 years	N/A	No association
Ambient concentration (?)	● In our subjects, no clear association between SO_2 concentrations and changes in spirometric tests[illegible]	5 years	4 Subjects	No association
Not given	● Pearson correlation coefficient between SO_2 levels and respiratory visits was negative.[illegible]	1 year	28 471 patients	No association

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Table 5E Epidemiology: Respiratory Health Effects Associated with Increases in Short-term Exposure to SO_2: Statistically Significant / Positive Findings – *Protective Effect*

Exposure Increase ppb (mg/m^3)	**Effect**	**Baseline Exposure (ppb)**	**Study Duration***	**Study Population**$^+$	**Reported Association**
38 ***(100)***	● Statistically significant protective effect against hospital admissions for asthma for adults >65	24 Hour mean: Amsterdam: 11 Rotterdam: 15	12 years	Respiratory admissions/d: 6.7	RR: (0.802 – 0.995)

Table 6. Respiratory Health Effects Associated with Short-term Exposure to SO_2 Summary of "Positive" Findings: Clinical and Non-clinical studies

Concentration (ppm)	Effects
0.03	◆*More rapid and more severe inflammatory response to influenza infection (to 0.1 ppm: Mice)*[illegible]
0.1	▲*Increased respiratory pause (Guinea pigs)*[259] ◆Slight reduction in FEV_1, $V_{max\ 50}$ (AC)[277] ◆Bronchoconstriction at lower concentrations in dry air than humidified air (HA)[097] ◆*Slight reduction in lung clearance (Rats)*[illegible] ◆*Increased antigen-specific antibodies in serum and bronchoalveolar fluid (Guinea pigs)*[illegible]
0.15	◆Decreased specific airway conductance (HA)[072]
0.2	◆Increased respiratory frequency (AA)[071] ●Bronchoconstriction (AA)[illegible]
0.25	◆Increased SRaw (AA)[118] ◆Increased bronchoconstriction(AA)[376]
0.3	◆Increased bronchoconstriction, returned to normal levels 30 min. post-exposure (and 0.6 ppm: AA)[097]
0.5	▲Increased SRaw (AA)[081,109] ▲Dose-dependent change in respiratory function (to 1 ppm: AA)[077] ▲*Dose-dependent increases in lung resistance (and 5 ppm: Rabbits)*[197] ◆Reduction in FEV_1, $V_{max\ 50}$, $V_{max\ 75}$ (AC)[099], (AA)[055] ◆Dose-dependent effect on FEV_1, $V_{max\ 50}$, $V_{max\ 75}$ (to 1 ppm: AA)[098] ◆Increased SRaw (AA)[061], greater with oral than nasal exposure (AA)[074]; with cold dry air (AA)[123] ◆SO_2 responsiveness decreased with opioid and increased with cyclooxygenase inhibitor (to 8 ppm: AA)[052] ◆Dryness, irritation, or burning of the throat (to 5 ppm: HA)[039] ◆Chest tightness, wheezing, dyspnea (and 1 ppm: AA)[064] ◆Increased bronchoconstriction(AA)[326] ●Changes in AM and BM chemotactic activity[319]
0.60	◆Decreased respiratory function (AA)[304,314] ●Bronchoconstriction[316] ●Increased SRaw(AA)[307]
0.75	▲Increased SRaw (HA)[060] ◆Reduction in FEV_1, increased total respiratory resistance (AC)[103] ◆Increased SRaw initially, decreased to pre-exposure levels after 1 hr of exposure (AA)[079] ◆Increase SRaw with hyperventilation[318] ◆Increased SRaw[304] ●Increased prevalence of compound cilia (HA)[040]
<1	Chest tightness, wheezing, dyspnea, cough (AA)[093]
1 ppm	▲Decreased spirometric function (HA)[096] ◆Reduction in FEV_1, $V_{max\ 50}$, $V_{max\ 75}$ (AC)[058,102], (AA)[110] ◆Slight reduction in FEV_1, $V_{max\ 50}$, $V_{max\ 75}$ or increased bronchoconstriction after exercise (HC)[042,311] ◆Increased SRaw (HA)[047], (AA)[062,064,078] ◆Decreased nasal mucous flow rate (HA)[063] ◆Decreased $MEF_{50\%VC}$ (HA)[070] ◆Discomfort proportional to SO_2 concentration (to 25 ppm: HA)[067] ◆Functional impairment of alveolar macrophages (to 5.0 ppm)[308] ◆*Increased respiratory resistance; decreased compliance (Guinea pigs)*[229] ◆*Dose-dependent increase in bronchoconstriction (to 2.5 ppm: Guinea pigs)*[245,246] ◆*Decreased proportion of macrophages in white cells (to 5 ppm: Guinea pigs)*[245] ■Threshold for bronchoconstriction (10 breaths;HA)[illegible] ●Decreased tidal volume, increased respiratory rate (HA)[illegible] ●Increased SRaw returning to control values post-exposure (HA)[illegible] ●Changes in SRaw[334] ●*Increased bronchial sensitivity (Dogs)*[illegible]
1.4	●*Increased intranasal transport time (Chickens)*[illegible]
1.5	●*Increased airway resistance (to 26 ppm: Guinea pigs)*[illegible]
2	◆Changes in SRaw (AA)[062,303] ◆Difference in ventilatory parameters between forced oral and free-breathing exposures (HA)[266] ●*Increased bronchial constriction (to 1000 ppm: Guinea pigs)*[316]

Table 6. Respiratory Health Effects Associated with Short-term Exposure to SO_2 Summary of "Positive" Findings: Clinical and Non-clinical studies (continued)

Concentration (ppm)	Effects
2.5	◆Decreased specific airway conductance greater with oral than nasal exposure (HA)[105] ◆Dose-dependent increase in ciliary beat frequency (to 12.5 ppm)[320,[illegible]] ●Dose-dependent decrease in ciliary beat frequency (12.5 ppm)[illegible] ●*Decrease in mucociliary activity (Guinea pigs)*[illegible] ●Decreased specific airway conductance (to 20 ppm; AA)[illegible]
3	●*Dose-dependent decrease in mucociliary activity (to 14 ppm; Guinea pigs)*[illegible]
3.4	◆*Increased incidence of pneumonia after exposure to SO_2 (to 34.5 ppm; Mice)*[182] ●*Increase in mononuclear and polymorphonuclear cells and number of plasma cells (to 18.5 ppm; Chickens)*[illegible]
4	◆Increased alveolar activity in BAL (and 8 ppm; HA)[083] ◆*Increased airway reactivity in asthmatic sheep 24 hr after exposure (Sheep)* [230] ●Decreased airway conductance and thoracic gas volume (HA)[illegible] ●Increased pulmonary flow resistance (HA)[illegible] ●Dose-dependent increase in mast cells, lymphocytes, macrophages in BAL (up to 8 ppm)[illegible] ●*Increased nasal turbinate clearance time (Chickens)*[illegible]
5 ppm	◆Decreased MMFR, increased bronchial clearance (HA)[045,056] ◆Decreased nasal mucous flow rate (HA)[048] ◆SRaw exhibited in all subjects[375] ●Increased bronchial reactivity (AA)[illegible] ●Decreased FEV_1 (to 11.5 ppm; AA)[illegible] ■Increased SRaw[illegible] ■*Dose-dependent decreased in ciliary beat frequency (to 12.5 ppm; Guinea pigs)*[illegible] ●*Dose-related increase in electrophoretic bands from nasal mucous (to 20 ppm; Rats)*[illegible]
6	◆*Inhibition of virus growth (Mice)*[238] ■*Decreased mucociliary transport rate (Chickens)*[illegible] ●*Decreased nasal mucous elastic recoil distance in vivo (Chickens)*[illegible]
7	●*Increased nasal and pulmonary flow resistance (to 230 ppm; Dogs)*[illegible]
8	▲*Increased lipid peroxide formation in lungs (Rats)*[177] ◆The effectiveness of atropine in controlling bronchoconstriction decreased as a subject's SO_2 responsiveness increased (HA,AA)[059] ◆Increases in macrophages, lymphocytes, and mast cells in BAL (HA)[090] ●Increased SRaw (AA)[illegible]
10 ppm	◆Bronchial obstruction, returned to control by 45-60 min. post-exposure (AA)[260] ◆*Increased airway reactivity in asthmatic sheep 24 hr after exposure (Sheep)* [231] ◆*Lesions of olfactory and respiratory epithelium (Mice)*[191] ◆*Increased concentrations of cholesterol, total lipids, gangliosides and decreased phospholipids (Guinea pigs)*[163] ◆*Decrease in thickness of olfactory mucosa, severe rhinitis (Mice)*[191] ◆*Inhibition of ciliary movement (Rabbits)*[468] ●*Ciliary movement stopped when SO_2 blown directly onto trachea (Rabbits)*[illegible] ●*Increased lung hypersensitivity to aerosolized methacholine (Dogs)*[illegible]
11	●*Bronchoconstriction (to 1000 ppm; Dogs)*[illegible]
15	◆Increased pulmonary flow resistance; greater from oral than nasal exposure (HA)[054] ◆*Dose-dependent increased in ciliary activity (to 77 ppm; guinea pigs)*[213]
16	◆SO_2 absorbed in upper respiratory tract (HA)[033]
17	◆*Dose-dependent respiratory depression (to 298ppm; Mice)*[243]
18	●*Decrease in turbinate clearance (Chickens)*[illegible]
20	◆*Delayed early clearance of upper respiratory tract (Rats)*[256] ■*Sneezing, rubbing eyes and noses (to 330, Guinea pigs)*[illegible] ●*Increased pulmonary flow resistance (Cats)*[illegible] ●*Edema, loss of cilia, epithelial thinning (Mice; 60 to 120 minutes)*[illegible]
27	●*Decrease in lung clearance (Hamsters)*[illegible]
40	◆*Dose-dependent decrease in % SO_2 retention, respiratory rate, minute volume, increase in tidal volume (Rats)*[253] ●*Epithelial damage in large airways (Hamsters)*[illegible] ●*Increase in static lung compliance (Mice)*[illegible]
50	▲*Reduction in pulmonary macrophage endocytosis(Hamsters)*[374] ◆*Reduced dynamic compliance (Dogs)*[189]

Table 6. Respiratory Health Effects Associated with Short-term Exposure to SO_2 Summary of "Positive" Findings: Clinical and Non-clinical studies (continued)

Concentration (ppm)	Effects
100 ppm	▲*Increase in minute volume (Chickens)*[183] ◆*Decreased glutathione concentration and inflammation (Rats)*[251] ●*Dose-dependent increase in lung resistance (to 1000ppm, Cats)*[illegible]
150	◆*Increased lung resistance, decreased breathing frequency (Rabbits)*[219]
200	◆*Decreased breathing frequency, increased tidal volume (Rabbits)*[244] ●*Increased inspiratory and decreased expiratory time (Rabbits)*[illegible] ●*Increased airway hyperreactivity (Dogs)*[illegible] ●*Decrease in mechanically stimulated cough reflex(Rabbits)*[illegible]
230	●*Increased numbers of polymorphonuclear leukocytes in tracheae(Rats)*[illegible]
300	●*Increased inspiratory and expiratory time (and 350 ppm, Rabbits)*[illegible] ●*Increased acid phosphatase and β-glucuronidase and β-galactosidase activity(Rats)*[illegible]
350	●*Decreased glycoprotein concentrations (Chickens)*[illegible]
400	●*Increase in bronchial response to histamine (Dogs)*[illegible]
500	▲*Decreased SRaw(Chickens)*[183] ◆*Changes to bioelectric properties and increased nonelectrolyte permeability (Dogs)*[156] ●*Changes in lung lipids and membrane permeability (Squirrels)*[illegible]
600	◆*Increased mucosal permeability (Rats)*[206] ◆*Increase in solid material recovered by bronchial lavage (Rats)*[196] ●*Acute bronchitis, bleeding of rhinopharynx, chronic tracheobronchial injuries (Rats)*[illegible]
627	●*Decreased surface forces and transpulmonary pressures (Rats)*[illegible]
800	◆*Reduction in minimal and maximal pulmonary surface tension (Rats)*[135] ◆*Gradient of decreasing damage in the tracheobronchial tree(Rats)*[165] ●*Loss of epithelial cells and increased permeability (Guinea pigs)*[illegible]
1000 ppm	▲*Initial decrease then increase in SRaw, increased respiratory frequency, decreased minute volume (Chickens)*[183]
1225	◆*Pulmonary edema, greater reduction in surface tension (Rats)*[131]
2500	●*Edema found in the separation of the surface epithelium from the alveolar septum (to 4000 ppm; Rats)*[illegible]
3000	●*Reduced tidal volume, increased respiratory frequency and pulmonary resistance (to 7000 ppm; Cats)*[illegible]

Table 7. Respiratory Health Effects Associated with Short-term Exposure to SO_2
Summary of "Negative" Findings: Clinical and Non-clinical studies

Concentration (ppm)	**Effects**
0.20	◆No effect on respiratory function in asthmatic adults [067]
0.4	◆Normal respiratory function in healthy adults [049,051]
0.5	▲No bronchoconstriction in healthy adults[073] ▲Normal respiratory function in asthmatic adults (and 0.25 ppm)[075]
0.60	◆No effect on healthy adults [009]
0.75	▲No effect on respiratory function in healthy adults [043]
0.8	◆No effect on respiratory function in asthmatic adults [101] ◆*No effect on respiratory function in guinea pigs* [294*]
1 ppm	◆No effect on respiratory function in healthy adults [040,112,306] ◆*No effect on respiratory function in guinea pigs* [257]
2	◆No effect on respiratory function in healthy adults [266] ◆No signs or symptoms observed in healthy adults[072] ◆*No biochemical changes observed in rat lungs*[223] ●*No effect on respiratory function in guinea pigs* [illegible]
3.3	◆No relationship between peak ambient SO_2 and hospital presentations or admissions for asthma[007] ?
3.6	◆No effect on respiratory function (from 1.1 ppm) in healthy adults [113]
4	●No effect on respiratory function in healthy adults [illegible]
5	◆No effect on respiratory function in healthy adults (from 0.5 ppm) [039]
10 ppm	
15	●No effect on FEV_1 in healthy adults [illegible]
20	●No effect on specific airway conductance in healthy adults (from 2.5 ppm)[illegible]
46.5	◆*No changes in pulmonary benzo(a)pyrene metabolism observed in rat lungs*[illegible]
50	●*No effect on water or histamine content of guinea pig lungs (and 10 ppm)*[illegible] ●*No significant changes in material in bronchoalveolar lavage fluid (Rats)*[illegible]
89	●*Few signs of respiratory distress in guinea pigs*[illegible]
100 ppm	◆*No change in bioelectric properties in dogs*[156]
300	●*No respiratory effects in donkeys (from 35 ppm)*[illegible] ●*No respiratory effects in rabbits (from 200 ppm)*[illegible]
700	●*No increased sensitivity to acetylcholine challenge (and 450, 600 ppm)*[144]
713	●*No respiratory effects in miniature donkeys (from 27 ppm)*[illegible]

Table 8. Non-respiratory Health Effects Associated with Short-term Exposure to SO_2

Summary of "Positive" Findings: Clinical and Non-clinical studies

Concentration (ppm)	Effects
0.01	◆Eye irritation reported in the general population during periods of high and low pollution up to 0.15 ppm[illegible]
0.03	◆*Antibodies to virus developed more rapidly and increased number of goblet cells in mice exposed up to 0.1 ppm for 4 weeks*[illegible]
0.1	▲*Enhanced ovalbumin-induced asthmatic reactions in guinea pigs exposed 5 hours/day for 5 days*[illegible] ◆*Increased blood pressure and heart rate observed in geese exposed to up to 400 ppb for 1 to 3 minutes*[illegible] ◆*Increased ovalbumin-specific antibodies and bronchoalveolar fluid in guinea pigs exposed to up to 16.6 ppm for 8hours/day, 5 days*
0.2	◆Differences in "total cardiac power" observed in healthy subjects exposed for 1 hour[071]
0.3	●Positive correlation between plasma S-sulfonate and atmospheric SO_2 in healthy smokers and non-smokers exposure to up to 6 ppm for up to 120 hours[illegible]
0.4	●Throat irritation and unpleasant smell reported in healthy adults exposed to between 0.4 and 4 ppm for 20 minutes[illegible]
0.5	▲Symptoms of decreased lung function with increased SO_2 concentration up to 0.5 ppm[077] ◆7 of 8 asthmatic subjects reported wheezing and chest tightness after 3 and 5 minutes of exposure[064]
0.75	◆Increased asthma symptoms reported in adults after 10 minutes of vigorous exercise during exposure[079]
0.87	◆*Decreased whole blood and packed cell viscosities after 24 hours exposure (Rats)*[illegible]
<1	◆Increased chest tightness, wheezing, cough, dyspnea in asthmatic subjects and taste and odour complaints from healthy subjects with increased SO_2 concentration during a 40 minute exposure[091]
1 ppm	▲Increased nose and throat irritation in healthy adults after exposure for 4 hours/day, 3 days/week for 3 weeks[096] ▲Shortness of breath, and chest discomfort in asthmatic subjects after 10 minutes of exposure[081] ◆Shortness of breath and wheezing reported in asthmatic children exposed for 30 minutes at rest and 10 minutes of exercise[041] ◆Discomfort proportional to SO_2 concentration reported in healthy adults exposed to up to 25 ppm SO_2 for 6 hours/day for 3 days[063] ◆2 of 8 asthmatic subjects reported chest tightness after 1 minute of exposure[064] ●Objective odours, irritation of the upper respiratory tract and unusual sensations in the lung reported by healthy adults exposure to between 1 and 60 ppm for 5 minutes[illegible]
1.8	●*Low, uniform [illegible] SO_2 concentration in heart muscle, high concentration in kidney, and low concentration in liver in dogs after exposures up to 148 ppm for 30 to 40 minutes (Dogs)*[illegible]
3	●Dose-dependent, reversible eye response observed in healthy adults exposed to between 3 and 60 ppm for 1 second[illegible]
3.4	●*Increased number of macrophages, lymphocytes, plasma cells and neutrophils in chickens exposed to up to 18.5 ppm for 1 to 14 days*[illegible]
4	◆Dose-dependent increased in macrophage activity 24 hours post-exposure up to 8 ppm in healthy adults[083] ●Increased macrophage activity in healthy adults 24 hours post exposure up to 11 ppm for 20 minutes[illegible]

Table 8, continued. Non-respiratory Health Effects Associated with Short-term Exposure to SO_2

Summary of "Positive" Findings: Clinical and Non-clinical studies

Concentration (ppm)	**Effects**
5	▲*Dose-dependent increase in plasma and liver triglycerides and HDL cholesterol in normal and hypertensive rats and decrease in plasma triglycerides, liver triglycerides and liver weight and increased in HDL cholesterol in diabetic rats exposed for up to 10 ppm for 15 days*[152] ▲*Changed behaviour in mice after exposure to up to 30 ppm for 24 days*[214] ◆50% decrease in nasal mucous flow in healthy adults after 4 hours of exposure[048] ◆*Changed behaviours in adult mice after prenatal exposure to up to 30 ppm for 14 days*[217] ◆*Decreased glutathione and varied enzyme activity observed in the hearts, liver, lung, and kidneys of rats exposed to up to 100 ppm for 5 hours/day for 7 to 28 days*[251] ●Threshold for tear production for 15 second exposure in humans ●*10% of inhaled SO_2 found in blood or plasma within first 30 minutes of exposure (up to 20 ppm; Rats)* ●*Increased frequencies of polychromatic erythrocyte formation (Mice)*
6	◆*Inhibition of influenza virus growth in mice exposed for 7 days*[258] ●*Increased hemoglobin at all concentrations up to 310 for 60 minutes exposure (Guinea pigs)*
10 ppm	▲*Increased mortality rate, decreased survival time (Mice)*[152] ▲*Decreased total lipids and free fatty acids, nasopharyngitis, somnolence, staggering, itching, preening, skin and eye irritation observed in guinea pigs exposed for 1 hr/day for 21 days*[159] ◆*Increased cholesterol, total lipids, phospholipids and decreased gangliosides observed in the hearts and other organs of guinea pigs exposed for 1 hour/day for 30 days*[183] ◆*Increased methemoglobin, sulfhemoglobin, lipoperoxidation and osmotic fragility, and decreased phospholipids and cholesterol after exposure for 1 hour/day for 30 days (Guinea pigs)*[236] ◆*Lipid content and enzyme activity vary depending on brain area in rats after one hour of exposure per day for 30 days*[249]
15	◆Coughing and burning sensations in the throat and substernal area reported by healthy adults exposed to 15 and 28 ppm for 10 minutes[054]
19	●*Inorganic sulphur in blood at concentrations up to 310 ppm at 60 minute exposures (Guinea pigs)*
20	◆*Decreased liver catalase activity (Rats)*[351]
23.5	●*Increased plasma and serum S-sulfonate levels in rabbits exposed for 14 to 62 hours*
25	◆*Decreased mean fetal body weight, delayed ossification of sternebrae and occipital bone after exposure on gestational days 6 to 15 (Mice)*[140]
40	◆*Reversible effects such as depressed feed and water intake, decreased body weight, and O_2 consumption observed in mice exposed to 4 to 11 days*[261] ◆*Decreased plasma thyroxine levels at 12 and 24 hours exposure; increased plasma glucocorticoids at 1 and 12 hours (Mice)*[212]
50	●*Decreased blood pressure in hypertension-resistant rats and increased blood pressure in other rats exposed to 6 hours/day, 5 days/week, for 6 weeks*
65	◆*Decreased pup weight in mice after exposure to 65 and 125 ppm during gestational days 7 to 17*[303]
70	◆*Minor skeletal variations in rabbits after exposure on gestational days 6 to 18*[149]
100 ppm	
200	●*Increased airway permeability to plasma proteins and cell shedding in dogs exposed for 2 hours*
250	◆*Increased uptake of Fe in airway epithelium after 3 hours exposure (Mice)*[308]
330	●*Hematoglutination observed in 5 of 10 guinea pigs exposed for 30 minutes*
400	●*3 of 30 exposed rats died in the first 5 hr of exposure; 22 of the remaining 27 died in the first week of exposures for 5 hr/day, 5 days/week* ●*Some signs of irritation observed in guinea pigs exposed for 30 minutes*
500	●*Decreased lipid levels and increased moisture content in squirrel hearts after 4 minute exposure*
590	●*Decreased survival time with increasing concentration up to 300,000 ppm (Rats)*
900	◆*% mortality rate increased with increased exposure time from 10 to 640 min (Mice)*[174]
1000 ppm	▲*2 of 10 chickens died at 60 min exposure*[183]
1400	◆*% mortality rate increased with increased exposure time from 10 to 640 min (Mice)*[174]
1900	◆*% mortality rate increased with increased exposure time from 10 to 640 min (Mice)*[174]
5000	▲*Decreased blood pH and O_2 and increased blood CO_2 in chickens exposed for 60 minutes; 9 of 10 chickens died at 60 min exposure*[183]

Table 9. Non-respiratory Health Effects Associated with Short-term Exposure to SO_2
Summary of "Negative" Findings: Clinical and Non-clinical studies

Concentration (ppm)	Effects
0.15	◆No signs or symptoms of irritation in health adults after exposure for 2 hours[072]
0.25	▲No signs or symptoms of irritation in asthmatic adults after exposure up to 0.5 ppm for 1 hour[075]
0.4	◆No signs or symptoms of irritation in asthmatic adults after exposure up to 1 ppm for 1 hour[101]
0.5	◆No correlation between plasma antioxidant concentrations and sensitivity to SO_2 in asthmatic adults exposed for 10 minutes[055]
0.95	◆*No change in mortality rates in mice exposed for 2 hours*[174]
1 ppm	▲No adverse eye effects observed in healthy adults exposed for 4 hours/day, 3 days/week for 3 week[096] ●*No significant effects on hemoglobin concentration (Rats)*
2	◆*No hematopoietic effects observed in rats exposed for up to 49 days*[225]
3.3	◆No association between peak SO_2 concentrations and hospital presentations of admissions for asthma, wheeze or shortness of breath up to 3.3 ppm[007]
8	●No effect observed on the cardiovascular system of healthy adults after exposures between 1 and 8 ppm for 10 minutes
10 ppm	
27	●*No difference in bacterial clearance rates in hamsters exposed for an undetermined amount of time*
30	▲*No changes observed in reproductive performance or neurobehavioural development of offspring in mice exposed to up to 30 ppm from 9 days pre-pregnancy to gestational day 12-14*[214]
40	●*No increased mortality in rats or hamsters with gradually increasing concentrations up to 400 ppm*
50	●No subjective eye effects reported by healthy adults exposed for 5 minutes
100 ppm	▲*No cardiovascular or hematopoietic effect on chickens during a 1 hour exposure*[183]
250	◆*No effect on number of dead or reabsorbed fetuses, no teratological effects in mice exposed to 32 to 250 ppm from gestational days 7 to 17*[203]
300	●*No signs of irritation in guinea pigs at concentrations lower than 300 ppm for a 30 minute exposure*
5000	◆*No change in blood pressure in rats exposure for 2 breaths*[211]

APPENDIX 1

TITLES AND AFFILIATIONS OF THE EXPERT PANEL

APPENDIX 1: TITLES AND AFFILIATION OF THE EXPERT PANEL

Randy P. Angle, PhD
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APPENDIX 2

MEMBERS OF THE REVIEW TEAM

APPENDIX 2: MEMBERS OF THE REVIEW TEAM

James Andruchow, M.Sc.

Lorelei Betke, M.Sc.

Michael Lam, Ph.D.

Norah Lee, M.Sc.

Rabindra Mahabeer, B.Sc.

Christine Teixiera, Ph.D.

Corinna Watt, M.Sc.

APPENDIX 3

SEARCH STRATEGY

APPENDIX 3: SEARCH STRATEGY

	Inclusion/Exclusion terms:	Number of documents identified:
1. TITLE or KEYWORDS must INCLUDE at least one of the following terms:	SO2; sulphur dioxide; sulfur dioxide; sulphur oxides; sulfur oxides	23499
2. From the list generated by (1), INCLUDE documents with any of the following terms in the TITLE or KEYWORDS:	Acute' subacute; short term; accident; case stud-; toxic; adverse; neurology-; CNS; central nervous system; nervous; brain; behaviour; clinical; teratol-; teratogen; embryotox; reproduct-; development; pregnan-; fetus; fetal; birth defect; chamber; respir; pulmonary; lung; asthma; irritant; airway; ocular; eye; trachea; nasal; bronch	6803
3. From the list generated by (2), EXCLUDE documents with any of the following terms in the TITLE or KEYWORDS	Cancer, Carcinogen, Tumour; Tumor; Neoplas-; Oncogen; Adenoma; Malignan-; Genotox; Mutat-; Mutagen; Cytogen; Clastogen; DNA repair; Aquatic; Marine; Benthic; Fish; Invertebrate; Cataly-; Computer; Treatment; Rain; Ecolog-; Magnesium; Mortality; Pine; Preservative; Kinetics; transport; deposition; lake; soil; bacteria; food, additives; economic; admission; distribution; aberrations; chroma-; calibrat-; pharmacokinetics; conversion; soil; precipitation; agriculture; woody; stability; crop; measurement; leaves; tree; forest; soybean; grass; algae; lichen; selenium	6802
4. From the list generated by (3), EXCLUDE documents with any of the following terms in the TITLE:	Air pollution; pulp mill; paper mill; ambient; vegetation; ecosystem; spectrophoto-; photosynthesis; occupational; fumigat-	3133
5. From the list generated by (4), ISOLATE by publication language:	English only	2468

APPENDIX 4: REVIEW FORMS

A. CLINICAL

B. NON-CLINICAL

C. EPIDEMIOLOGY

CLINICAL REVIEW FORM

Author:	**Internal ID:** ID of study within database
Title:	
Year:	
Abstract:	
Objective: The authors' stated objective and identification of relevant experiments in documents reporting findings from multiple studies.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
						No observed adverse effect level	Lowest observed adverse effect level

Observations:

Reported data
*Statistically significant

Author's Conclusions:

Quotations of the author's primary conclusions

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	✎ Is the number of subjects sufficient to produce statistically significant results? ✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ✎ Are the subjects a representative sample of the population to which extrapolations will be made? ✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? ✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?

B. Exposure conditions:	✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? ✎ Was there a gradient of exposure levels? ✎ Were the chosen exposure levels appropriate to investigate the primary objective? ✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ✎ Were both nominal and actual exposure concentrations recorded? ✎ Was the duration of exposure appropriate to investigate the primary objective? ✎ Was the duration of exposure precisely defined? ✎ Was the mode of administration appropriate? ✎ Was the frequency of administration appropriate to investigate the primary objective? ✎ Was evidence provided to justify the dosing regime? ✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined ✎ Was a full description of the monitoring devices provided? ✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	✎ Were the subjects randomly assigned to exposure groups? ✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) ✎ Was a control group assayed? ✎ Is the control regime appropriate? ✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) ✎ Was the length of follow-up appropriate? ✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	✎ Were appropriate endpoints examined? ✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) ✎ Were accepted assessment methods employed? ✎ Were assessment measures sufficiently sensitive? ✎ Was the nature and severity of symptoms graded against an explicitly defined scale? ✎ Did qualified personnel conduct the exams? ✎ Was the timing and frequency of assessment logical? ✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? ✎ Was individual data reported for each subject? ✎ Are all withdrawals listed, with the reason for withdrawal?

F. Data analysis:	✎ Are the statistical methods used appropriate? ✎ Are confidence intervals reported? ✎ Did the investigator appropriately interpret the statistics? ✎ Are the observed trends statistically significant?
G. Interpretations:	✎ Was the original objective addressed? ✎ Are the results novel? ✎ Was the design of the study appropriate to investigate the primary objective? ✎ Were assumptions reasonable? ✎ Are the methods reproducible? ✎ Did either the study design or the statistical analysis limit confounding variables? ✎ Do the conclusions make biological sense? ✎ Are the extrapolations reasonable? ✎ Is the paper in a peer-reviewed journal? ✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:
Reviewer's summary of study findings and interpretation of the toxicological significance and clinical relevance of these findings.
Confidence index:
Reviewer's assessment of the quality of the study design, conduct and reporting.

Review – Confidence index ranking:

High
High to moderate
Moderate to high
Moderate
Moderate to low
Low to moderate
Low

NON-CLINICAL REVIEW FORM

Author:	**Internal ID:** ID of study within database
Title:	
Year:	
Abstract:	
Objective: The authors' stated objective and identification of relevant experiments in documents reporting findings from multiple studies.	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
								No observed adverse effect level	Lowest observed adverse effect level

Observations:

Reported data
*Statistically significant

Author's Conclusions:

Quotations of the author's primary conclusions

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	✎ Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level: if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? a) Specie

	☐ *Guideline: Rat, or justification for use of another mammalian specie.* b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* c) Sex d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* e) Weight variation ☐ *Guideline: Below 20% variation* f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? ✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* ✎ Were the chosen exposure levels appropriate to investigate the primary objective? ✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ✎ Were both nominal and actual exposure concentrations recorded? ✎ Was the duration of exposure appropriate to investigate the primary objective? ✎ Was the duration of exposure precisely defined? ✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* ✎ Was the mode of administration appropriate? ✎ Was the frequency of administration appropriate to investigate the primary objective? ✎ Was evidence provided to justify the dosing regime? ✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers

	appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* ✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* ✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* ✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* ✎ Was a full description of the monitoring devices provided? ✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* ✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ✎ Were test animals randomly assigned to exposure groups? ✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) ✎ Was a control group assayed? ✎ Is the control regime appropriate? ✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	✎ Were appropriate endpoints examined? ✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and

	dose-response relationship was provided for each symptom)? ✎ Were accepted assessment methods employed? ✎ Were assessment measures sufficiently sensitive? ✎ Was the nature and severity of symptoms graded against an explicitly defined scale? ✎ Did qualified personnel conduct the exams? ✎ Was the timing and frequency of assessment logical? ✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? ✎ Was individual data reported for each test animal?
G. Data analysis:	✎ Are the statistical methods used appropriate? ✎ Are confidence intervals reported? ✎ Did the investigator appropriately interpret the statistics? ✎ Are the observed trends statistically significant?
H. Interpretations:	✎ Was the original objective addressed? ✎ Are the results novel? ✎ Was the design of the study appropriate to investigate the primary objective? ✎ Were assumptions reasonable? ✎ Are the methods reproducible? ✎ Did either the study design or the statistical analysis limit confounding variables? ✎ Do the conclusions make biological sense? ✎ Are the extrapolations reasonable? ✎ Is the paper in a peer-reviewed journal? ✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Reviewer's summary of study findings and interpretation of the toxicological significance and clinical relevance of these findings.

Confidence index:

Reviewer's assessment of the quality of the study design, conduct and reporting.

Review – Confidence index ranking:

- High
- High to moderate
- Moderate to high
- Moderate
- Moderate to low
- Low to moderate
- Low

EPIDEMIOLOGY REVIEW FORM

Author:	**Internal ID:** ID of study within database
Title:	
Year:	
Abstract:	
Objective: The authors' stated objective and identification of relevant experiments in documents reporting findings from multiple studies.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL

Observations:

Reported data
*Statistically significant

Author's Conclusions:

Quotations of the author's primary conclusions

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	✎ Is the number of subjects sufficient to produce statistically significant results? ✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ✎ Are the subjects a representative sample of the population to which extrapolations will be made? ✎ Were identical 'criteria for exclusion' applied to both cases and controls? ✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	✎ Was the exposure concentration measured? ✎ Is the exposure duration known?
C. Equipment:	✎ Was a full description of the monitoring devices provided? ✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	✎ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? ✎ Were appropriate controls recruited? ✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) ✎ Was the timing and length of follow-up appropriate? ✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	✎ Was the assessment identical for cases and controls? ✎ Were appropriate endpoints examined? ✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) ✎ Were accepted assessment methods employed? ✎ Were assessment measures sufficiently sensitive? ✎ Was the nature and severity of symptoms graded against an explicitly defined scale? ✎ Did qualified personnel conduct the exams? ✎ Was the timing and frequency of assessment logical? ✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? ✎ Was individual data reported for each subject? ✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	✎ Are the statistical methods used appropriate?

	✎ Are confidence intervals reported? ✎ Did the investigator appropriately interpret the statistics? ✎ Are the observed trends statistically significant?
G. Interpretations:	✎ Was the original objective addressed? ✎ Are the results novel? ✎ Was the design of the study appropriate to investigate the primary objective? ✎ Were assumptions reasonable? ✎ Are the methods reproducible? ✎ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? ✎ Do the conclusions make biological sense? ✎ Are the extrapolations reasonable? ✎ Is the paper in a peer-reviewed journal? ✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Reviewer's summary of study findings and interpretation of the toxicological significance and clinical relevance of these findings.

Confidence index:

Reviewer's assessment of the quality of the study design, conduct and reporting.

Review – Confidence index ranking:

High
High to moderate
Moderate to high
Moderate
Moderate to low
Low to moderate
Low

APPENDIX 5

TABULAR SUMMARY OF STUDIES

Mortality **p.196**
Respiratory – functional **p.202**
Respiratory – biochemical **p.224**
Respiratory – structural **p.227**
Respiratory – signs and symptoms **p.229**
Respiratory – other **p.230**
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Mortality

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
Non-clinical					
▲172	Azoulay-Depuis et al., 1982	10 ppm	1 to 3 wk	Mice	Increased mortality rate, decreased survival time
◆174	Grose et al., 1986	0.95 ppm	2 hr	Mice	No change in mortality rates
◆224	Bitron and Ahronson, 1978	900, 1400 and 1900 ppm	10 to 640 min	Mice	% mortality rate increased with increased exposure time
●284	Hilado and Machado, 1977	3000 to 6800 ppm	5 to 30 min	Mice	LC_{50} 5 min 6800 ppm 10 min 4400 ppm 15 min 4000 ppm 30 min 3000 ppm
●198	Asmundsson et al., 1973	400 ppm 40-400 ppm (gradual inc.)	5 hr/d, d 5/wk, 6 wk	Rats Rats and hamsters	3 of 30 died in 1st 5 hr; 22 of remaining 27 died in 1st wk No increased mortality with gradually increasing concentrations
●218	Cohen et al., 1973	224 to 500,000 ppm	Until death or up to 4 hr	Rat	Decreased survival time with increased concentration above 590 ppm
▲183	Fedde and Kuhlmann, 1978	1000 ppm 5000 ppm	60 min	Chickens	2 of 10 birds died 9 of 10 birds died
Epidemiology *– All-cause or total mortality*					
◆336	Katsouyanni et al., 1997	38ppb increase		General population	3% increase daily mortality Western European cities 1% increase daily mortality in central eastern European cities
◆338	Xu et al., 1994	doubling of SO_2 concentrations mean: 38 ppb	One year	General population	11% increase all-cause mortality Beijing, China

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
		max: 240ppb			
◆345	Sunyer et al., 1996	38ppb increase	1985- 1991	General population	Statistically associated with total, elder & cardio mortality for whole year; same plus respiratory mortality for summer
◆349	Touloumi et al., 1996	38ppb increase	1987-1991	General population	12% increase in daily mortality risks
◆351	Dab et al., 1996	Mean daily 1 hr max = 23 ppb 24 hr = 41 ppb		General population	Significant associations with daily counts of death
◆352	Zmirou et al., 1996	19ppb increase	1985 - 1990	General population	Significant association with total mortality minus external causes, respiratory and cardiovascular deaths
◆361	Touloumi et al., 1994	10% reduction in SO_2	1984 - 1988	General population	0.65% decrease in daily mortality
◆403	Wietlisbach et al., 1996	3 day moving average	1984-1989	General population	Significant associations with total mortality minus external causes, respiratory deaths and cardio deaths
◆464	Wong et al., 2001	Average 6.5 ppb (cool season) and 6.9 ppb (warm season)		General population	Significant associations with mortality in the cool season, but not the warm season
●012	Buechley et al., 1973	190ppb	Daily average	General population	2% excess mortality when SO_2 conc. greater than 190 ppb
●334	Moolgavkar et al., 1995	100 ppb increase	1973 – 1988 Daily	General population	Significantly associated with daily mortality in the spring and winter, but not fall and summer

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
●337	Spix et al., 1993	Increase from 9 ppb to 355 ppb	1980 - 1989	General population	10% excess mortality
●348	Spix and Wichmann, 1996	Mean 8 and 47 ppb	Daily	General population	Increase in daily mortality of 3% for lag day
●357	Glasser and Greenburg, 1971	Increase in ambient SO_2 0.20 ppm or less and days with 0.4 ppm or more	1960-64	General population	Increases in mortality, independent of weather factors in New York City
●359	Krzyzanowski and Wojtyniak, 1991	Daily max 229 ppb	Winter months 1977- 1989	General population	Significance is not established for association between air pollution and daily mortality in Cracow, Poland
●366	Schimmel and Greenburg, 1972	Air pollution	1963 -1968	General population	20% of excess deaths in New York City
●391	Rahlenbeck and Kahl, 1996	38ppb	1981-1989 winters	General population	4.5% excess mortality in East Berlin
●395	Burnett et al., 1998	0.7 – 10.5 ppb	Daily	General Population	1.4% average increased risk of mortality over 11 Canadian cites from changes in mean SO_2 concentrations
●407	Le Tertre et al., 2002	19ppb increase	1990-1995	General population	Significant associations between SO_2 increase and total, cardiovascular and respiratory mortality in 9 French cities
●408	Ha et al., 2003	7.8 ppb increase		General population	Some positive associations with mortality for some age groups
●414	Botter et al., 2002	4 ppb increase	1991-1993	People over 65 years old	Significant 2.4% increase in daily death count for people over 65 in Sao Paulo, Brazil for a 3 day lag

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
●419	Schwartz et al., 2001	4ppb increase		General population	Weak association between increase and daily deaths (0.27%, 95% CI; 0.18-0.73%) in eight Spanish cities
●434	Vedal et al., 2003	0.3-15 ppb	summer	General population	Small but significant increase in percentage total deaths was observed with a standard deviation increase in summer in Vancouver, B.C. Similar observations were made in the winter for lag 1.
●465	Odriozola et al., 1998	38ppb increase		General population	Significant associations between increase in SO_2 concentration and daily mortality
Epidemiology – *Respiratory Mortality*					
◆027	Vigotti et al., 1996	1 to 316 ppb	Daily changes over 10 y	General population	Risk of respiratory death increased with increased SO_2 concentration (RR: 1.12, 95%CI 1.03, 1.23)
◆430	Zeghoun et al., 2001	IQR increase 7-14 ppb – Rouen, France IQR increase 4-13 ppb Le Havre, France		General population	Associations with respiratory mortality in Rouen
●002	Derriennic et al., 1989	19 to 25 ppb	Daily averages	General population	Statistically significant association between daily SO_2 conc. and respiratory deaths
●412	Hong et al., 1999b	Above 40 ppb	Jan 1995 – Aug 1996	General population	Significant predictor of respiratory mortality with lag day 1, but not with total or cardiovascular mortality in Inchon, Korea
●422	Wong et al., 2002	4 ppb increase		General	Barely significant associations in

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
				population	respiratory mortalities
●440	Bobak and Leon, 1992	Range <5 - >22 ppb		Infant	Statistically significant association post neonatal respiratory mortality weak, non-significant associations for other infant mortalities
●461	Venners et al., 2003	38 ppb	One year	General population	Statistically significant associations between respiratory and cardiovascular mortality in Chongqing, China -strongest on second and third lag days
Epidemiology – *Stroke Mortality*					
●397	Hong et al., 2002b	17.43 ppb	Jan 1991 – Dec 1997	General population	Significant increased risk of ischemic stroke mortality. Hemorrhagic stroke mortality not significant.
●415	Hong et al., 2002a	5.7 ppb increase	2 day lag	General population	2.9% (95% CI: 0.8%-%.0%) increase in stroke in Seoul, Korea
◆337	Verhoeff et al., 1996	38 ppb increase	1986 - 1192	General population	No association found between air pollution and daily mortality in Amsterdam regardless of lag day
◆458	Simpson et al., 1997	60 ppb	Maximum hourly	General population	No significant association in daily mortality in Amsterdam
●332	Mazumdar et al., 1982	0.38 ppm increase	1958-1972 winters - daily	General population	No significant associations observed between daily deaths/concentrations of smoke and SO_2 in London, England
●350	Wojtyniak and Pickarski, 1996	Range of medians 11 to 28 ppb		General population	Inconsistent associations between SO_2 concentrations and cardiovascular mortalities in four Polish cities
●354	Bacharova et al., 1996	Mean: 5 to 16 ppb	1987-1991 Daily	General population	No significant association between $S0_2$ concentrations and daily number of deaths

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
		Mean: 4 – 187 ppb			in Bratislava, Slovak Republic
●355	Ballester et al., 1996	4 ppb increase	1991 – 1993 Daily	General population	No significant results reported
●400	Ballester et al., 2002	4 ppb increase		General population (13 Spanish cities)	Single city analysis – no statistically significant associations between $S0_2$ and daily mortality. Cities combined – associated with a 0.5% increase in daily deaths
●356	Mackenbach, 1993	Range: 5-9 ppb		General population	Positive regression coefficient for the effect of SO_2 on mortality dwindles to zero when all potential confounding factors are taken into account
●365	Anderson et al., 1996	7-17 ppb	1987-1992 warm season	General population (London)	Significant association between increased ambient $S0_2$ concentrations and all-cause mortality
●389	Kelsall et al., 1997	12.9 ppb increase		General population	Non-significant associations in SO_2 concentrations and total mortality.
●442	Saldiva et al., 1994	Mean: 6 ± 4 ppb	May 1990 – Apr 1991	Children (Sao, Paulo, Brazil)	No association between SO_2 concentrations and respiratory mortality
●443	Kinney and Ozkaynak, 1991	Mean 15 ± 6 ppb	`970 – 1979	General population	No association between changes in SO_2 concentration and mortality in Los Angeles County, California
●479	Kotesovec et al., 2000	38 ppb increase	Daily	General population (Northern Bohemia)	No association between daily total mortality when gender, ad, and cause of death were not separated out.

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
●480	Hong et al., 1999a	4 ppb increase	Daily	General population (Inchon, Korea)	Not significant for either total or cardiovascular mortality
●483	Schwartz and Dockery, 1992	38 ppb increase		General Population (Philadelphia)	Signification positive association between total mortality and SO_2 for both current day and prior day SO_2 measurements. Total mortality estimated to increase by 5% with each 38 ppb increase in So_2.
***Epidemiology** – Case Studies*					
●021	Harkonen et al., 1983	"High"	~ 20 to 25 min	Healthy adults	Death in 2 of 9 men
●270	Charan et al., 1979	"High"	Unknown	Workers	2 of 5 men died; accidental mine explosion

Respiratory System-Functional

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
Clinical - Adolescents					
◆038	Koenig et al., 1982a	1 ppm	30 min rest; 10 min exercise	Adolescents with hyperactive airways	Reduction in FEV_1, V_{max50}, $V_{max\ 75}$
◆042	Koenig et al., 1982b	1 ppm	30 min rest; 10 min exercise	Healthy, adolescents	Slight reduction in FEV_1, V_{max50}, $V_{max\ 75}$ after exercise
◆099	Koenig et al., 1985	0.5 ppm	50 min	Asthmatic, adolescents	Reduction in FEV_1, V_{max50}, $V_{max\ 75}$
◆103	Koenig et al., 1987	0.75 ppm	10 min	Asthmatic, adolescents	Decreased FEV_1; increased total respiratory resistance
◆102	Koenig et al., 1990a	1 ppm	10 min	Asthmatic, adolescents	Decreased FEV_1; increased total respiratory resistance

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
◆277	Koenig et al., 1990b	100 ppb	15 min	Asthmatic, adolescents	Slight decrease in FEV_1 and V_{max50}
Clinical - *Effects observed-healthy subjects*					
▲060	Stacy et al., 1981	0.75 ppm	2 hours	Healthy adults	Increased SRaw
▲096	Kulle et al., 1986	1 ppm	4 hr/d; 3d/wk, 3wk	Healthy adults	Decreased spirometric function
◆045	Newhouse et al., 1978	5 ppm	2.5 hr	Healthy adults	Decreased MMFR, increased bronchial clearance
◆047	Bedi et al., 1984	1 to 2 ppm	2 hours	Healthy adults	Increased SRaw
◆056	Wolff et al., 1975	5 ppm	3 hr	Healthy adults	Decreased MMF
◆070	Snell and Luchsinger, 1969	0.5, 1, 5 ppm	15 min	Healthy adults	Decreased $MEF_{50\%VC}$ at 1 and 5 ppm
◆072	Kagawa, 1983	0.15 ppm	2 hr	Healthy adults	Decreased specific airway conductance; increased residual capacity and residual volume
◆063	Andersen et al., 1974	1, 5, 25 ppm	6 hr/d; 3 d	Healthy adults	Decreased nasal mucus flow rate; increased nasal airflow resistance
◆318	Islam, et al., 1994	0.73 ± 0.05 00m	5 minutes	Healthy non-smoking adults	Statistically greater increase in SRaw after hyperventilation with SO_2
◆048	Andersen et al., 1977	5 ppm	4 hr	Healthy adults	Decreased nasal mucus flow rate in anterior nose
●069	Nadel et al., 1965	4 to 6 ppm	10 min	Healthy adults	Decreased airway conductance and thoracic gas volume
●076	Frank et al., 1964	1-2, 4-6, 14-17 ppm	30 min	Healthy adults	Increased pulmonary flow resistance at 4-6 and 14-17 ppm
●323	Frank et al., 1961	1,5,15 ppm		Healthy male adults	Most results were not significant and there was substantial variability among the volunteers

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
●121	Douglas and Coe, 1987	1 ppm	Eyes: 15 s Lungs: 10 breaths	Healthy adults	Threshold for bronchoconstriction
●032	Amdur et al., 1953	1 to 8 ppm	10 min	Healthy adults	Decreased tidal volume; increased respiratory rate
●317	Lawther et al., 1975	1 to 30 ppm	10 min to one hour	Healthy adults	Normal breathing at 1ppm – no significant changes Deep breathing at 3 ppm – no significant changes Hyperventilation of 1 ppm (undetermined period of time) – small but significant changes in SRaw Quiet breathing of 10,15,20 and 30 ppm for 10 min at each concentration – significant changes for most
●324	Sim and Pattle, 1957	Maximum 2160 mg min/m^3 (mask) and 3620 mg min/m^3 (chamber)		Healthy male adults	800 mg min/m^3 – no effects Above 1300 mg min/m^3 – resistance to air flow significantly increased in half the volunteers exposed by mask and chamber. At this dosage and higher, high pitched musical rales observed
●416	Whittenberger and Frank, 1963	1, 5 and 13 ppm	Unidentified	Work colleagues	Increased specific airway resistance
Clinical - No effects observed: healthy subjects					
▲043	Stacy et al., 1981	0.75 ppm	4 hr	Healthy adults	No effect
◆039	Kreisman et al., 1976	0.5 to 5 ppm	1 to 5 min	Healthy adults	No effect

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
◆051	Bedi et al., 1979	0.4 ppm	2 hr	Healthy adults	No effect
◆049	Bedi et al., 1982	0.4 ppm	2 hr	Healthy adults	No effect
◆122	Folinsbee et al., 1985	1 ppm	2 hr	Healthy adults	No effect
◆040	Kulle et al., 1984	1 ppm	4 hr/day, 3 d/wk, 3 wk	Healthy adults	No effect
●087	Sandstrom et al., 1988	0.4 to 4 ppm	20 min	Healthy adults	No effect
●325	Lawther, 1955	0, 5,10,20 ppm	10 min each by nose and mouth	Healthy male adults	No effect
Clinical - No effect healthy subjects: effects asthmatics					
▲073	Jaeger et al., 1979	0.5 ppm	3 hr	Normal, asthmatic	Normal: no effect Asthmatic: decreased MMF
◆306	Schachter et al., 1984	0,0.25,0.50, 0.75 and 1.0 ppm	40 min	Normal, asthmatic	Healthy subjects – no pulmonary function changes at all concentrations or in asthmatics below 1.0 ppm At 1.0 ppm significant changes in SRaw and FEV_1 at max flow at 50% of vital capacity
◆309	Linn et al., 1987	0,0.2,0.4 and 0.6 ppm	1 hr including three 10 min exercise periods	Normal, atopic, asthmatic	Normal and Atopic – little response at all levels Moderate to severe asthmatics - increasing response with increased dose
●092	Tan et al., 1982	2.5 to 20 ppm	5 min	Normal, asthmatic	Normal: no effect Asthmatic: decreased specific airway conductance

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
●108	Harries et al., 1981	Up to 15 ppm	Not clear	Normal, asthmatic	Normal: no effect; Asthmatic: decreased FEV_1 at 5 to 11.5 ppm
Clinical – *Effects observed – asthmatic subjects*					
▲077	Gong et al., 1995	0.5, 1 ppm	10 min	Asthmatic adults	Dose-dependent changes in pulmonary function parameters
▲081	Roger et al., 1985	0.25, 0.5, 1 ppm	75 min	Asthmatic adults	Increased SRaw at 0.5 and 1 ppm
▲109	Jorres and Magnussen, 1990	0.5, 0.75 ppm	30 min	Asthmatic adults	Change in SRaw
◆055	Trenga et al., 1999	0.5 ppm	10 min	Asthmatic adults	Decreased FEV_1
◆064	Balmes et al., 1987	0.5 or 1 ppm	1, 3, 5 min	Asthmatic adults	Small increases in SRaw; wheezing, tightness of chest, dyspnea
◆071	Tunnicliffe et al., 2001	200 ppb	1 hr	Asthmatic adults	Increased respiratory frequency
◆375	Sheppard et al., 1980	1,3, 5ppm	10 min	Asthmatic adults	Increased SRaw occurred at lower concentrations (1 ppm) in mild asthmatics At 5 ppm all asthmatics exhibited significant increases in SRaw
◆062	Tam et al., 1988	2 ppm 4 ppm	4 min 10 min	Asthmatic adults	Increased dyspnea, wheezing, SRaw
◆078	Kehrl et al., 1987	1 ppm	1 hr	Asthmatic adults	Increased SRaw
◆098	McManus et al., 1989	0.5 or 1 ppm	30 min	Asthmatic adults	Dose-dependent effect on FEV_1, total airway resistance, V_{max50}, V_{max75}
◆110	Heath et al., 1994	1 ppm	20 min	Asthmatic adults	Decreased V_{max50}, R_T, FEV_1
◆118	Bethel et al., 1985	0.25 ppm	10 min	Asthmatic adults	Increased SRaw
◆303	Horstman et al., 1986	0.25,0.5,1.0,2.0 ppm	10 min	Asthmatic adults	Substantial variability observed in bronchial sensitivity SRaw 100% greater than the response

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
					to clean air ranged between 1.28 and 1.90 ppm for 23 subjects – for remaining subjects greater than 2.0 ppm
◆304	Linn et al., 1983 a	0.75 ppm	10 min (chamber) -once with encumbered breathing, once with nose clips	Asthmatic adults	Greater increase in SRaw observed upon exposure to SO_2 than clean air exposure Excess increase significantly greater with mouthpiece than with unencumbered breathing
◆310	Linn et al., 1983 b	0,0.2,0.4 and 0.6 ppm	5 min	Asthmatic adults	Dose-response effect with only the changes at 0.6 ppm being highly significant
◆311	Horstman et al., 1988	1.0 ppm		Asthmatic adult	During mild exercise, significant increases in bronchoconstriction occurred at 2.0 minutes of exposure
◆326	Bethel et al., 1983	0.5 ppm	5 min	Asthmatic adults	Bronchoconstriction observed during moderate to a greater degree with heavy exercise when breathing through mouthpiece and during heavy exercise when breathing through a facemask.
◆376	Sheppard et al., 1981b	010,02.5,0.50 and 1 ppm (mouthpiece)	5-10 min	Mild Asthmatics	Significant bronchoconstriction observed for most at 0.25 ppm
●084	Wolff et al., 1984	5 ppm	2.5 hr	Asthmatic adults	Increased bronchial reactivity
●116	Fine et al., 1987	8 ppm	Up to 1 min	Asthmatic adults	Increased SRaw

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
●316	Linn et al., 1984 c	0.6 ppm for 6 hour periods on two successive days	1 hr	Asthmatic adults	Volunteers exercised heavily for 5 min at beginning of exposures and after 5 hours of exposure. Substantial bronchoconstrictive response were observed only immediately after exercise
Clinical - *No effects observed: asthmatic subjects*					
▲075	Bailey et al., 1982	0.25, 0.5 ppm	1 hr	Asthmatic adults	No effect
◆067	Devalia et al., 1994	200 ppb	6 hr	Asthmatic adults	No effect
◆101	Linn et al., 1985b	Up to 0.8 ppm	1 hr	Asthmatic adults	No effect
Clinical - *Effect and recovery*					
◆061	Sheppard et al., 1983	0.5 ppm	3 min, 3 x 30 min intervals	Asthmatic adults	Increase SRaw in first exposure; less increase in second & third exposures
◆079	Hackney et al., 1984	0.75 ppm	3 hr	Asthmatic adults	Increased SRaw initially; decreased to pre-exposure levels after 1 hr of exposure
◆097	Linn et al., 1998	0.3, 0.6 ppm	10 min	Asthmatic adults	Increased bronchoconstriction; returned to normal by 30 min post-exposure
◆260	Gokemeijer et al., 1973	10 ppm	3 min	Asthmatic adults	Bronchial obstruction; returned to control by 45-60 min post-exposure
●053	Toyama and Nakamura, 1964	1 to 60 ppm	5 min	Healthy adults	Increased SRaw returning to control values post-exposure
Clinical - *Nose vs. mouth exposure*					
◆054	Speizer and Frank, 1966b	15 or 28 ppm	10 min	Healthy adults	Increased pulmonary flow resistance; more from oral than nasal exposure
◆074	Kirkpatrick et al., 1982	0.5 ppm	5 min	Asthmatic adults	Increased SRaw; greater with oral than nasal exposure

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
◆105	Melville, 1970	2.5 to 10 ppm	10 min to 1 hr	Healthy adults	Decreased specific airway conductance; greater effect with oral than nasal exposure
◆266	Bedi and Horvath, 1989	2 ppm	30 min	Healthy adults	Difference in ventilatory parameters between forced oral and free-breathing exposures
Clinical - *Temperature and humidity*					
◆057	Sheppard et al., 1984	0.1 ppm	3 min	Healthy adults	Bronchoconstriction at lower concentrations in dry air than humidified air
◆123	Bethel et al., 1984	0.5 ppm	3 min	Healthy and asthmatic adults	Increased SRaw with SO_2 in cold dry air with asthmatics
◆314	Linn et al., 1984 a	0, 0.3 and 0.6 ppm Temperatures 21°C, 7°C and -6°C - constant humidity of 80%	5 min	Asthmatic adults	Considerable variability – cold seemed to exacerbate the overall response to SO_2
●313	Linn et al., 1984 b	0, 0.2,0.4 nd 0.4 ppm 85% (high) and 50% (low) relative humidity	5 min heavy exercise	Asthmatic adults	Bronchoconstriction increased with increasing SO_2 concentrations, but did not vary significantly with humidity
◆307	Linn et al., 1985a	0.6 ppm 21°C and 38°C humidity 20%	5 min heavy exercise	Asthmatic adults	Greater effects on SRaw observed at low temperature and low humidity

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
***Non** clinical - Effects observed-bronchial clearance*					
◆235	Ferin and Leach, 1973	0.1 ppm 1 ppm	70 hr 170 hr	Rats	Slight decrease in lung clearance
◆256	Mannix et al., 1983	20 ppm	4 hr	Rats	Delayed early clearance of upper respiratory tract
◆213	Oomichi and Kita, 1974	15, 32, 58, 77 ppm	2 to 6 min	Guinea pigs	Dose-dependent decrease in ciliary activity
●132	Riechelmann et al., 1995	3, 6, 9, 11, 14 ppm	30 min	Guinea pigs	Dose-dependent decrease in mucociliary activity
●164	Knorst et al., 1994	2.5, 5, 7.5, 10, 12.5 ppm	30 min	Guinea pigs	Decrease in mucociliary activity at 2.5 ppm; Dose-dependent decrease in ciliary beat frequency above 5 ppm
●134	Trimpe et al., 1986	27±3 ppm	35 d	Hamsters	Decrease in lung clearance
●129	Wakabayashi et al., 1977	1.4 to 66 ppm	16hr/d, 7 d	Chickens	Increased intranasal transport time
●137	Ukai et al., 1984	18 to 40 ppm	1 hr/d, 7 d	Chickens	Decrease in turbinate clearance
●138	Ukai et al., 1983	4 to 40 ppm	1 hr, 4x/d for 2d	Chickens	Increases in nasal turbinate clearance time
●149	Majima et al., 1985	6 ppm	16 hr/d, 7 d	Chickens	Decrease in mucociliary transport rate
***Non-clinical** - Effects observed-bronchoconstriction/specific airway resistance*					
▲183	Fedde and Kuhlman, 1978	100 ppm 500 ppm 1000 ppm	60 min	Chickens	Increase in minute volume Decreased SRaw Initial decrease then increase in SRaw; increased respiratory frequency, decreased minute volume
▲197	Barthelmy et al., 1988	0.5 or 5 ppm	45 min	Rabbits	Dose-dependent increases in lung resistance
◆244	Davenport et al.,	200 to 400 ppm	15 to 20 min	Rabbits	Decreased breathing frequency;

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
	1984				increased tidal volume
●194	Citterio et al., 1985b	300 to 350 ppm	Unreported	Rabbits	Increased inspiratory and expiratory time
●234	Davies et al., 1978a	200 ppm	10 min	Rabbits	Increased inspiratory time; decreased expiratory time
▲259	Park et al., 2001	0.1 ppm	5 hr/d, 5 d	Guinea pigs	Increased respiratory pause
◆189	Atzori et al., 1992	50 to 500 ppm	15 min	Guinea pigs	Reduced dynamic compliance
◆229	Amdur et al., 1983	1 ppm	1hr	Guinea pigs	Increased respiratory resistance; decreased compliance
◆245	Halinen et al., 2000a	1, 2.5, 5	10 min each, consecutive	Guinea pigs	Dose-dependent increase in bronchoconstriction at 1 and 2.5 ppm
◆246	Halinen et al., 2000b	1 ppm	1 hr	Guinea pigs	Weaker effects than previous study
●216	Amdur, 1959	2 to 1000 ppm 24 ppm	1 hr 3 hr	Guinea pigs	Increased bronchial constriction
●227	Amdur and Underhill, 1970	1.5 to 26 ppm	1 or 2 hr	Guinea pigs	Increased airway resistance
◆243	Alarie et al., 1973	17, 32, 62, 89, 123, 198, 298 ppm	10 min	Mice	Dose-dependent respiratory depression
◆253	Leong and MacFarland, 1965	40, 64, 83, 145, 231, 426, 751 ppm	2 hr	Rats	Dose-dependent decrease in % SO_2 retention, respiratory rate, minute volume; increase in tidal volume
●167	Cho et al., 1968	11 to 1000 ppm	0.1 to 6 min	Dogs	Bronchoconstriction at all levels
●170	Frank et al., 1965	7 to 230 ppm	15 to 20 min	Dogs	Increased nasal and pulmonary flow resistance
●258	Lewis and	10 and 30 ppm	5 min	Dogs	Increased lung hypersensitivity to

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
	Kirchner, 1984				aerosolized methacholine
●190	Eady and Jackson, 1989	400 ppm	2 hours	Dogs	Increase in bronchial response to histamine
●162	Islam et al., 1972	1, 2, 5, 10 ppm	3x60-min periods	Dogs	Increased bronchial sensitivity
●146	Norris and Jackson, 1989	200 ppm	2 hr	Dogs	Increased airway hyperreactivity
●170	Frank and Speizer, 1965	7-16 ppm, 25-34 ppm or 60-61 ppm	20 min	Dogs	Increased nasal and pulmonary flow resistance
●186	Grunstein et al., 1977	3000 to 7000 ppm	24 to 40 seconds	Cats	Reduced tidal volume, increased respiratory frequency and pulmonary resistance
●290	Corn et al., 1972	15-25 or 30-40 ppm	30 min	Cats	Pulmonary flow resistance – lower then 20 ppm no response; at 20ppm only one animal showed a response
●372	Thompson et al., 1990	100,500,800 and 1000 ppm	1,5,10 breaths	Cats	Concentration dependent response was observed in lung resistance with the administration of 10 breaths of 100 – 1000 ppm
◆230	Abraham et al., 1981	5 ppm	4 hr	Sheep, normal and allergic	Increased airway reactivity in asthmatic sheep 24 hr after exposure
◆231	Abraham et al., 1980	5 and 10 ppm	4 hr	Sheep, normal and allergic	Increased airway reactivity in both sheep 24 hr after exposure to 10 ppm
●205	Spiegelman et al., 1968	27 to 713 ppm	30 min	Miniature donkeys	No effects
◆191	Giddens and Fairchild, 1972	10 ppm	4 to 72 hr	Mice	Lesions of olfactory and respiratory epithelium

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
◆207	Ukai, 1977	0.03 to 0.1 ppm	4 wk	Mice	More rapid and more severe inflammatory response to influenza infection
◆238	Fairchild, 1977	6 ppm	7 d	Mice	Inhibition of virus growth
●300	Hanacek, 1987	200-300 ppm	10-15 min	Rabbits	Decrease in mechanically stimulated cough excitability and cough reflex strength
***Non Clinical** - No effects observed*					
◆204	Amdur et al., 1978	0.2, 0.4, 0.8 ppm	2 hr	Guinea pigs	No effects
◆257	McJilton et al., 1976	1 ppm	2x60 min	Guinea pigs	No effects
●226	Amdur and Underhill, 1968	2 ppm	10 min	Guinea pigs	No effects
●263	Lippman et al., 1975	35 to 300 ppm	30 min	Donkeys	No effects
●161	Hanacek et al., 1991	200 to 300 ppm	10 to 20 min	Rabbits	No effects
Epidemiology - Children					
◆005	Boezen et al., 1999	Approx. 0.22 to 23 ppb (ambient)	Unknown	Asthmatic children	Children with bronchial hyperresponsiveness and high serum IgE concentrations are more susceptible to increases in air pollution
◆013	Dockery et al., 1982	64 to 174 ppb (ambient)	SO_2 spikes over 2-year period	Children	Slight decrease in pulmonary function with increasing SO_2 conc.
◆018	Hoek and Brunekreef, 1993	Ambient, range unknown	SO_2 spikes over three	Children	Decreased FVC, FEV1, MMFR with SO_2 concentrations > 38 ppb

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
			winters		
◆426	Schwartz et al., 1994	Ambient, range unknown	Unknown	Children	Not significantly associated with cough incidence or upper respiratory symptoms Lower respiratory symptoms above concentrations of 22 ppb
◆448	Segala et al., 1998	Ranged from 1.7 to 32 ppb Mean: 8.3 ± 5.1 ppb	Same Day Lag day 1	Asthmatics (Paris)	Significant increase in incidence of asthma attack in mild asthmatics with an increase of 19 ppb
◆449	Roemer et al., 1993	40 ppb and 56 ppb	24 hr average and 1 hr max	Children (The Netherlands)	Small but statistically significant negative association between SO_2 concentrations for both morning and evening peak flow
●362	Agocs et al., 1997	Unknown	Unknown	Asthmatic children (Budapest, Hungary)	Longitudinal study of lung peak expiratory flow rates and ambient air pollution – no consistent, significant association
●385	Romieu et al., 1995	Unknown	Unknown	Children (Mexico City)	Statistically significant associations between total number of emergency visits for respiratory disease and levels of SO_2 on the same day Associations between number of emergency visits for asthma or total number of emergency visits and SO_2 concentration were not statistically significant.
●394	Lin et al., 2003	Unknown	Lagged over	Children	Significant association between

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
			6 or 7 days	(Toronto, Ontario)	asthma hospitalization and exposure to SO_2 in girls aged six to twelve, but not in boys
●398	Lee et al., 2002	4.4 ppb increase (ambient)	Unknown	Children (South Korea)	Statistically significant association between hospital admission for asthma
●413	Delfino et al., 2003	Increases in ambient		Hispanic children (Los Angeles)	Significant associations between bothersome and more severe asthma symptoms with increases in ambient SO_2
●456	Chew et al., 1999	7.6 ppb increase	Lagged by 1 or 2 days	Children (Singapore)	Significant positive correlation between 7.6 ppb increase in SO_2 levels lagged by 1 or days and daily asthma emergency room visits
●469	Hajat et al., 1999	6.8 ppb change in ambient levels		General population	Statistically significant association between GP consultations for asthma and other lower respiratory diseases in children. No significant findings reported for adults and the elderly.
●432	Mortimer et al., 2001		2 day moving average lag increase	Asthmatic children (4-9) (USA)	Association between a 2-day moving average lab increase in SO_2 and morning asthma symptoms
●485	Garty et al., 1998			Asthmatic children	Positive correlations between emergency room visits for acute asthma attacks and ambient mean SO_2 concentrations.

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
●435	Peters et al., 1996	51 ppb increase		Children (East Germany and Czech Republic)	Weak association between 51 ppb SO_2 increases and decreases peak expiratory flow
●445	Queiros et al., 1990		Monthly Quarterly	Children (Oporto area of Portugal)	Very small significant correlations between monthly and quarterly mean ambient SO_2 concentrations and asthmatic attacks
●450	Braun-Fahrlander et al , 1992	Range 11-27 (ambient)	Unknown	Children (two cities in Switzerland)	No statistically significant associations between ambient levels and respiratory symptoms
●451	Henry et al., 1991	Ambient	Unknown	Children (Two towns, New South Wales, Australia)	No significant association between ambient SO_2 levels and asthma symptoms
●457	Kieding et al., 1995	Unknown	Unknown	Children	No association between SO_2 levels and number of total contacts or contacts for respiratory illness with the Copenhagen Emergency Medical Service in children.
●462	Yu et al., 2000	Unknown	Unknown	Children (Seattle., Washington)	No significant association between ambient SO_2 concentrations and asthma symptoms
●467	Roemer et al., 1998	Unknown	Morning Evening	Asthmatic children	No clear association between SO_2 and morning or evening PEF
Epidemiology – *Hospital admissions or other incidence of chronic obstructive pulmonary disease (COPD)*					
◆351	Dab et al., 1996	Mean 11 ppb Mean 23ppb	24 hour 1 hr max	General population (Paris)	Significantly associated with admission for COPD for same-day exposure
◆369	Anderson et al.,	19 ppb increase		General population	Varied considerably across the cities

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
	1997	in daily mean		(Europe)	(Amsterdam, Barcelona, Paris, Rotterdam); not statistically significant for all ages In the warm season, borderline significant results between hospital admissions for COPD and a 19 ppb increase in daily means SO_2 levels with inconsistent lags of either the same day or day 2
◆406 ◆402	Desqueyroux et al., 2002a,b	0.76 to 10 ppb (mean: 2.7 ± 1.9 ppb) 1.1 to 31 ppb (mean: 7.3±4.6 ppb)	Summer Winter		No association between physician-monitored exacerbation of COPD symptoms or asthma
◆431	Tenias et al., 2002	4ppb increase		General population (Valencia, Spain)	Not associated with emergency room visits for COPD
●439	Sunyer et al., 1991	38ppb (daily mean)		General population (Barcelona)	Small but statistically significant association between daily number of emergency room admissions and daily levels of SO_2 Increase of 38 ppb daily means led to an average of 2 additional admissions per day
●437	Sunyer et al., 1993	9.5 ppb increase (ambient)	5 year period winter and summer	General population (Barcelona)	Resulted in 6% increase in emergency admissions in winter and 9% in summer

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
●481	Wong et al., 1999	4ppb increase (ambient)		General population	Weak and barely significant associations between increase of 4 ppb in ambient SO_2 levels and hospital admissions for respiratory disease and COPD
Epidemiology *– Hospital admission or clinic visits for respiratory disease and/or asthma*					
◆367	Bates and Sizto, 1987	2.21 to 5.14 ppb (winter) 1.65 to 3.97 (summer)	Jan, Feb, Jul and Aug 1974 and 1976-1983	General population (southern Ontario)	Significant correlations were found between SO_2 and deviations form the mean respiratory admissions for day of the week, season and year.
◆340	Walters et al., 1994	15ppb and 48 ppb		General population (Birmingham, UK)	Daily SO_2 levels were weakly, but significantly associated with hospital admissions for respiratory diseases for the same day in the summer and with a two-day lag in the winter.
◆423	Wong et al., 2002	4 ppb increase		General population (Hong Kong and London, England)	Asthma admissions were not significantly associated with in either city. Respiratory admissions was small, but significant in Hog Kong
●342	Emerson, 1973	Atmospheric conditions and air pollution	82 weeks, weekly	32 Volunteers	SO_2 was reported to be significantly correlated with FEV_1 in one volunteer and with MEFR in two volunteers
●346	Ponce de Leon et al., 1996		1987-1992	General Population (London)	Weak and questionably significant associations were reported between an increase in SO_2 concentrations from the 10th to the 90th percentile

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
					and two different age groups and two seasons.
●347	Ponka and Virtanen, 1996b	Mean 5-10 ppb	24 hour	General population	Positive associations – asthma admissions
●393	Hwang and Chan, 2002	1.5 to 16.9 ppb mean: 5.4±3.0 ppb		General population (Taiwan)	Significant associations between current day SO_2 concentrations and daily number of clinic visits for lower respiratory illness People over 65 most susceptible and associations decreased as lag day increased
●399	Martins et al., 2002		Six day	General population (Sao Paulo, Brazil)	Significant association between a six-day moving average of SO_2 and emergency room visits for chronic lower respiratory disease in the elderly
●409	Jaffe et al., 2003	19 ppb increase	Daily	Asthmatics (Cincinnati, Cleveland, and Columbus, Ohio)	12% increased risk of an emergency department episode with a 19 ppb increase in SO_2 across all cities
●410	Hajat et al., 2002	5.7 and 7.8 ppb		General population (London, England)	Significant increases in the number of physician consultations for upper respiratory disease for adults and those 14 years and younger Elderly – no significant changes
●428	Pinter et al., 1996			General population	Significant correlations between daily concentrations of SO_2 and incidence of acute respiratory morbidity
●444	Hoek and	Mean		General population	Small significant positive

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
	Brunekreef, 1994	5.7±5.5ppb		(Netherlands)	associations between previous day SO_2 concentrations and daily respiratory symptoms, but no pulmonary function.
●471	Schwartz, 1995			General population (two cities, USA)	SO_2 levels were significant predictors of hospital admissions for respiratory disease with very different ratios of SO_2-to-PM. The lag day differed between two cities
●472	Peters et al., 1997	25 ppb increase (5 day mean levels of SO2)	Evening	General population (Czech Republic)	Weak, but statistically significant decrease in evening peak expiratory flow.
Epidemiology – *Other asthma incidence*					
◆333	Moseholm et al., 1993	6.5 to 6.8 ppm	24 hr means 8-month period	Asthmatics	Lung function was associated with ambient SO_2 concentrations of SO_2, as well as with temperature, relative humidity, and medicine intake Increased SO_2 concentrations corresponded to decreased peak flow levels above 15ppb
●364	Buchdahl et al., 1996		Daily		Significant associations between variations in daily SO_2 concentrations and the incidence of acute wheezy episodes, after adjustment for season
●433	Tarlo et al., 2001	Ambient	March to November		
●455	Neukirch et al.,	19 ppb increase	5 day lag	Asthmatics	Significant associations and incidence

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
	1998		(winter)	(Paris)	of wheeze and nocturnal cough SO_2 levels correlated significantly with decreased morning peak expiratory flow
Epidemiology – *Other hospital admissions*					
●453	Ponka, 1991		Daily		Significant correlations of daily concentrations of SO_2 and admissions to emergency wards in the elderly
Epidemiology – *No associations observed*					
◆023	Kesten et al., 1995	0 and 0.15 ppm	Year Long	General population	No association was observed between SO_2 and emergency room visits Some association with other air pollutants
●331	Moolgavkar et al., 1997	Unknown	1986-1991	General population (Minneapolis St. Paul, Minnesota and Birmingham, Alabama)	No significant associations between hospital admissions and SO_2 concentrations
●353	Schouten et al., 1996	11ppb – Amsterdam 15 ppb – Rotterdam	24 hour mean	General population (Amsterdam and Rotterdam)	Results inconsistent with both negative and positive associations observed between SO_2 concentrations and hospital admissions for respiratory disease.
●425	Tenias et al., 1998	10 ppb	24 hour mean	General population (Valencia, Spain)	No significant associations between SO_2 levels and emergency room visits for asthma in an ecological study as part of the APHEA project
●454	Burnett et al., 1999		Daily	General population	No statistically significant associations between daily hospital

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
					admissions for respiratory and daily measures of SO_2
●470	Harre et al., 1997	(ambient)	Morning Evening peak	General population (Christchurch, New Zealand)	No significant association between ambient SO_2 levels in either morning or evening peak expiratory flow rate.
●473	Prescott et al., 1998	10 ppb increase (moving average)	3 days	General population (Edinburgh)	No statistically significant change in the risk of hospital admissions
●475	Hernandez-Garduno et al., 1997	Unknown	Unknown	Patients (clinics, Mexico City)	Ambient So_2 levels negatively correlated with patient visits to clinics in Mexico City
●478	Holmen et al., 1996	Measurements 10 m off the ground	Daily		No statistically significant correlations between emergency department visits for asthma and daily ambient SO_2 levels.
●482	Sheppard et al., 1999	10 ppb increase (ambient)		General population (Seattle)	No significant associations between a 10ppb increase in ambient SO_2 levels and hospital admissions for asthma
●484	Castellague et al., 1995			General population (Barcelona Spain)	No statistically significant associations between ambient SO_2 levels and emergency room visits
Epidemiology - *Workers*					
◆007	Donoghue and Thomas, 1999	Up to 3300 ppb (ambient)	Unknown	Asthmatics	No relationship between peak ambient SO_2 and hospital presentations or admissions for asthma
◆009	Zuskin et al., 2000	Up to 190 ppb (ambient)	Unknown	Outdoor workers *vs.* indoor controls	Increased prevalence of upper airway symptoms in outdoor workers

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
◆016	Holness et al., 1985	Average: 0.47 ppm	Occupational exposure	Nickel smelter workers	Higher prevalence of cough, dyspnea, decreased FVC, FEV_1 over workweek
●017	Likas et al., 2001	0.15 to 0.66 ppm	Occupational exposure	Greenhouse workers	No effects on lung function
●029 ●030 ●031	Lawther et al., 1974 a, b, c)	FEV_1 MMF Peak expiratory flow	Every working for five years	Four normal subjects (central London)	MMF showed most consistent association with pollution levels Respiratory infections had a substantial effect on pulmonary measurements Outdoor exercise had some association with decreases in FEV_1 and MMF None of the associations reported to be significant
Epidemiology – *Case reports*					
●021	Harkonen et al., 1983	Unknown (Accidental exposure)	Approx. 20 to 25 min	Healthy adults	Death in 2 of 9 men; in survivors: thoracic pain, coughing, decreased FVC, FEV_1, MMFR, bronchial hyperreactivity
◆001	Piirila et al., 1996	Unknown (Accidental exposure)	Approx. 20 to 25 min	13-year follow-up of Harkonen et al., 1983	Bronchial hypersensitivity in all patients; obstructive ventilatory impairment
◆272	Rabinovitch et al., 1989	Unknown (Accidental exposure)	Unknown	2 miners	Severe airway obstruction, hypoxemia, active inflammation three weeks after accident
●269	Woodford et al., 1979	Unknown (Accidental	Unknown	Previously healthy young male	Rhinorrhea, cough, pulmonary edema, pulmonary obstructive

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
		exposure)			syndrome
●271	Galea, 1964	Unknown (Accidental exposure)	Unknown	35-year old male	Cough, dyspnea, wheezing, death 17 days after exposure

Respiratory System-Biochemical

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
Clinical					
◆033	Speizer and Frank, 1966a	Approx. 16 ppm	Unreported	Adults	SO_2 absorbed in the upper respiratory tract
◆052	Field et al., 1996	0.5 to 8 ppm	Unreported	Asthmatic adults	SO_2 responsiveness decreased with opioid and increased with cyclooxygenase inhibitor
◆066	Bechtold et al., 1993	1 or 7 ppm	10 to 20 min every other day for 3 wk	Asthmatic adults	S-sulfonate conc. In nasal lavage fluid is a potential short-term biomarker of exposure to SO_2
◆083	Sandstrom et al., 1989a	4 and 8ppm	20 min	Healthy adults	Increased alveolar activity in bronchoalveolar lavage fluid (BAL)
◆090	Sandstrom et al., 1989b	8 ppm	20 min	Healthy adults	Increases in macrophages, lymphocytes, and mast cells in BAL
●091	Sandstrom et al., 1989c	4, 5, 8, 11 ppm	20 min	Healthy adults	Dose-dependent increase in mast cells, lymphocytes, macrophages in BAL up to 8 ppm
◆085	Witek and Schachter, 1985	1 ppm	40 min	Asthmatic adults	Correlation between increased dose of SO_2 and dose of methacholine required
◆321	Lazarus et al., 1997	8.0 ppm	4 min	Asthmatic	Leukotriene receptor antagonist

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
					zafirlukast inhibited SO_2 induced bronchoconstriction in 12 subjects
Non-clinical					
◆133	Riedel et al., 1988	0.1 to 16.6 ppm	8 hr/d, 5 d	Guinea pigs	Increased antigen-specific antibodies in serum and bronchoalveolar fluid
◆163	Haider, 1985	10 ppm	1 hr/d, 30 d	Guinea pigs	Increased conc. Cholesterol, total lipids, gangliosides; decreased phospholipids
◆178	Atson et al., 1991	250 ppm	10 min	Guinea pigs	No attenuation of SO_2-induced bronchoconstriction with inhalation of two different medications
◆245	Halinen et al., 2000	1, 2.5, 5 ppm	10 min	Guinea pigs	Decreased proportion of macrophages in white cells
●370	Hajj et al., 1996	500,100,1500 and 2000 ppm		Guinea pigs	Tachykinin release from sensory endings does play a role in SO_2 induced bronchoconstriction
●452	Ito et al., 1995	800 ppm	2 hrs	Guinea pigs	Direct epithelial injury from SO_2 inhalation results in loss of epithelial cells and an increase in permeability
◆155	Kahana and Aronovitch, 1968	800 ppm 1225 ppm	1 hr 2 hr	Rats	Reduction in minimal and maximal pulmonary surface tension Pulmonary edema, greater reduction in surface tension
◆206	Vai et al., 1980	600 ppm	30 to 100 hr	Rats	Increased mucosal permeability
◆251	Langley-Evans et al., 1996	5, 50, 100 ppm	5 hr/d, 7 to 28 d	Rats	Decreased glutathione conc. And inflammation at 100 ppm
◆252	Husain and Dehnen, 1978	46.5 ppm	up to 4 wk	Rats	No change in benzo(a)pyrene metabolism

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
●181	Barry and Mawdesley-Thomas, 1970	300 ppm	6 hr/d, 10 d	Rats	Increased acid phosphatase and β-glucuronidase, and β-galactosidase activity
●193	Grause and Barker, 1978	5 to 20 ppm	7 d	Rats	Dose-related increase in electrophoretic bands from nasal mucous
●262	Kahana and Aronovitch, 1966	627-751 ppm 257-450 ppm	1 hr 9 x 4 hr	Rats	Decreased surface forces and transpulmonary pressures Results unclear
◆207	Ukai, 1977	0.03 to 0.1 ppm	4 wk	Mice	Increased response to viral challenge
▲374	Skornik and Brain, 1990	50 ppm		Hamsters	Significant reduction observed after 40 minutes of continuous running while breathing 50 ppm SO_2 No changes with non-exercise, between exercise with SO_2 and no exercise without SO_2
●147	Rana et al., 1979	500 ppm	5 min	Squirrels	Changes in lung lipids and membrane permeability
●149	Majima et al., 1985	6 ppm	16 hr/d, 7 d	Chickens	Decreased nasal mucous elastic recoil distance *in vivo*
●199	Okuyama et al., 1979	3.4 to 18.5 ppm	1 to 14 d	Chickens	Increased in mononuclear and polymorphonuclear cells, and number of plasma cells
●221	Bauer, 1981	350 to 400 ppm	3 hr	Chickens	Decreased glycoprotein conc.
◆150	Man et al., 1986	100 ppm 500 ppm	75 min	Dogs	No change in bioelectric properties Changes to bioelectric properties and increased nonelectrolyte permeability

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
◆225	Azoulay et al., 1980	2 ppm	1 to 49 d	Rats	No changes observed

Respiratory Effects - Structural

Clinical					
◆320	Kienast et al., 1994a	2.5 to 12.5 ppm at 37°C and 100% humidity	30 min		Dose-dependent decrease in ciliary beat frequency observed from low to high concentrations
●427	Kienast et al., 1996	0, 2.5, 5.0, 7.5, 10.0 and 12.5 ppm	30 min	12 healthy volunteers	Dose-dependent decrease in ciliary beat frequency observed
●466	Riechelmann et al., 1994	2.5, 5, 7.5, 10, and 12. 5 ppm	30 and 120 min		Concentration-dependent reduction in ciliary beat frequency in human nasal cells
●046	Carson et al., 1985	0.75 ppm	2 hr	Normal adults	Increased prevalence of compound cilia
Non-clinical					
●132	Riechelmann et al., 1995	3, 6, 9, 14 ppm	30 min	Guinea pigs	Dose-dependent decrease in mucociliary activity, but only minor morphological changes
◆250	Knauss et al., 1976	600 and 700 ppm	3 hr/day for 9, 18, or 30 hrs	Rats	Increase in solid material recovered by bronchial lavage
◆305	Stratmann et al., 1991	800 ppm	8 hrs	Rats	Gradient of decreasing damage in the tracheobronchial tree in the peripheral direction Most severe lesions in trachea epithelium

●166	Gross et al., 1969	2500 and 4000 ppm	15 min	Rats	Edema found in the separation of the surface epithelium from the alveolar septum
●210	Pariente, 1980	600 ppm 1000 ppm	100 hr 4 hr	Rats	Acute bronchitis, bleeding of rhinopharynx, chronic tracheobronchial injuries
●447	Hong, 1996	30 to 50 ppm	4 or 12 hr	Rats	No significant changes in cell count, LDH, total protein, CC16, and lysozyme bronchoalveolar lavage fluid
●477	Farone et al., 1995	230 ppm	1 day	Rats	Substantially increased numbers of polymorphonuclear leukocytes in tracheas
◆191	Giddens and Fairchild, 1972	10 ppm	4 to 72 hr	Mice	Decrease in thickness of olfactory mucosa, severe rhinitis
●208	Weiss and Weiss, 1976	40 ppm	6 to 9 d	Mice	Increase in static lung compliance
●287	Min et al., 1994	20 ppm increased	30 to 60 to 120 min	Mice	60 – 120 minutes - Injuries included edema, loss of cilia, epithelial thinning, and epithelial desquamation 30 minutes – no changes
●198	Asmundsson et al., 1973	40, 100, 200, 250, 400 ppm	5 hr/d, 5 d/wk, 6 wk	Hamsters	Epithelial damage in large airways after one week
◆468	Blanquart et al., 1995	10 and 30 ppm	1 hr	Rabbits	Ciliary activity was significantly inhibited
●294	Dalhamn and Strandberg, 1961	200 ppm	45 min	Rabbits	Ciliary movement stopped when 10ppm SO_2 or greater was blown directly onto the trachea
●417	Strandberg, 1964	0.05 to 700 ppm		Rabbits	Differences in the absorption of high concentrations of SO_2 and low

					concentrations in the respiratory tract
●286	Frank et al., 1967	22±2 ppm	30 to 60 min	Dogs	Investigation of uptake of SO_2 into body fluids
●418	Balchum et al., 1959	1.1-141 ppm	20 to 40 minutes	Dogs	Investigation of uptake of SO_2 into body fluids
◆308	Knorst et al., 1996a	1.0,25. and 5.0 ppm	30 min	Human in vitro	Functional impairment of human alveolar macrophages after exposure
●319	Knorst et al., 1996b	0.5, 1.5 and 2.5 ppm	15 min	Human in vitro	Changes in AM and BM chemotactic activity Cell viability not affected

Respiratory System – Signs and Symptoms

Clinical – *Effects observed*					
◆039	Kreisman et al., 1976	0.5 to 5 ppm	1 to 5 min	Healthy adults	Dryness, irritation or burning of the throat
◆063	Andersen et al., 1974	1 to 25 ppm	6 hr/d, 3 d	Healthy adults	Discomfort proportional to SO_2 conc.
◆064	Balmes et al., 1987	0.5 or 1.0 ppm	1, 3, or 5 min	Asthmatic adults	Chest tightness, wheezing, dyspnea
◆093	Witek et al., 1985	less than 1 ppm	40 min	Health and asthmatic adults	Chest tightness, wheezing, dyspnea, cough
Clinical – *No effects observed*					
◆072	Kagawa, 1983	0.15	2 ppm	Healthy adults	No effects observed
Non-clinical studies					
●125	Amdur, 1954	89 ppm	8 to 16 hr	Guinea pigs	Few signs of respiratory distress
●142	Matsumura, 1970	20, 60, 180, 330 ppm	30 min	Guinea pigs	Sneezing, rubbing eyes and noses, uneasiness at 330 ppm
●144	Matsumura et al., 1972	450, 600, 700 ppm	30 min	Guinea pigs	No increased sensitivity to acetylcholine challenge
Epidemiology – *Effects observed*					

◆011	Cohen et al., 1974	0.11 to 0.15 ppm	Peak hourly conc.	Healthy adults and children	Increased reports of eye and throat irritation, chest discomfort, shortness of breath, restricted activity, medical visits
◆010	Carnow et al., 1969	up to 0.30 ppm	10 months	Adults with chronic bronchopulmonary disease	Dose-response association between SO_2 conc. greater than 0.25 ppm and percent person-days of illness
◆016	Holness et al., 1985	0.47 ppm	Average	Nickel smelter workers	Higher prevalence of cough, dyspnea, lower baseline function over the workweek
Epidemiology – *No effects observed*					
◆015	Love et al., 1982	4 to 46 ppm	Annual mean ambient conc.	Elementary school children	No effects
◆018	Hoek and Brunekreef, 1993	up to 38 ppb	Winter	Children	No effects
●004	Franklin et al., 1985	Not reported	10 d	Asthmatic and non-asthmatic children	No effects
●006	Ayres et al., 1989	Polluted area: 19-40 ppb Non-polluted area: 13-27 ppb	Unreported	General population	No effects
●267	NIOSH, 1984	Up to 1.03; 0.2 to 1.8 ppm	Unreported	Cement plant workers	Shortness of breath, frequent colds, cough, sore throat, chest tightness; association with SO_2 exposure unclear

Respiratory System - Other

◆182	Fairchild et al.,	3.4 to 34.5 ppm	7 d	Mice	Increased incidence of pneumonia

	1972				after exposure to SO_2
●126	Suzuki, 1969	10 and 50 ppm	3 hr	Guinea pigs	No effect on water or histamine content of lungs
●169	Frank et al., 1969	1 to 50 ppm	1.5 to 5 min	Dogs	Most inhaled SO_2 is absorbed in the nose

Signs and Symptoms

Clinical – *Effects observed – healthy subjects*					
▲096	Kulle et al., 1986	1 ppm	4 hr/d, 3 d/wk, 3 wk	Healthy adults	Increased nose and throat irritation
◆054	Speizer and Frank, 1966b	15 and 28 ppm	10 min	Healthy adults	Coughing, burning sensations in throat and substernal area
◆063	Andersen et al., 1974	1 to 25 ppm	6 hr/d, 3 d	Healthy adults	Some discomfort, proportional to SO_2 concentration
●053	Toyama and Nakamura, 1964	1 to 60 ppm	5 min	Healthy adults	Reported objective odours, irritation of upper respiratory tract and unusual sensations in lung
●087	Sandstrom et al., 1988	0.4 to 4 ppm	20 min	Healthy adults	Irritation of throat, unpleasant smell
Clinical – *Effects observed – asthmatic subjects*					
▲077	Gong et al., 1995	Up to 0.5 ppm	1 hr	Asthmatic adults	Decreased lung function with increased SO_2 conc.
◆064	Balmes et al., 1987	0.5 and 1 ppm	1, 3, and 5 min	Asthmatic adults	2 of 8 subjects reported chest tightness at 1 ppm for 1 min; 7 of 8 reported wheezing and chest tightness after 0.5 ppm for 3 and 5 min
◆079	Hackney et al., 1984	0.75 ppm	3 hr	Asthmatic adults	Increased asthma symptoms after 10 minutes of vigorous exercise, decreasing to normal by 1 hr post-exposure

◆041	Koenig et al., 1981	1 ppm	30 min at rest, 10 min exercise	Asthmatic children	Shortness of breath, wheezing
●087	Roger et al., 1985	1 ppm	10 min	Asthmatic young male	Shortness f breath and chest discomfort Trend towards increased wheezing, deep breathing discomfort, and cough
Clinical – *Effects observed – healthy and asthmatic subjects*					
◆093	Witek et al., 1985	< 1 ppm	40 min	Healthy and asthmatic adults	Increased chest tightness, wheezing, cough, dyspnea in asthmatics and taste and odour complaints from healthy subjects with increased SO_2 concentration
Clinical – *No effects observed*					
▲075	Bailey et al., 1982	0.25, 0.5 ppm	1 hr	Asthmatic adults	None observed
◆072	Kagawa, 1983	0.15 ppm	2 hr	Healthy adults	No symptoms observed
◆101	Linn et al., 1985b	0.4, 0.8, 1 ppm	1 hr	Chronic pulmonary obstructive disease	No observed effects
Clinical – *Eye Symptoms*					
●121	Douglas and Coe, 1987	3 to 60 ppm	Eye: 1 s Lung: 10 breaths	Healthy adults	Eye: dose-dependent, reversible response
Non-clinical					
▲159	Haider et al., 1981	10 ppm	1 hr/d, 21 d	Guinea pigs	Nasopharyngitis, somnolence, staggering, itching, preening, skin and eye irritation
◆261	Johnson et al., 1972	40 ppm	4 to 11 d	Mice	Reversible effects: depressed feed and water intake, decreased body weight and O_2 consumption
●142	Matsumura, 1970a	20, 60, 180, 300	30 min	Guinea pig	No signs of irritation lower than 300

		ppm			ppm
●143	Matsumura, 1970b	400 ppm	30 min	Guinea pig	Some signs of irritation
Epidemiology					
◆007	Donoghue and Thomas, 1999	Up to 3300 ppb	Peak concentrations over a 3 yr period	Asthmatic population	No association between peak SO_2 concentrations and hospital presentations or admissions for asthma, wheeze or shortness of breath
◆011	Cohen et al., 1974	0.01 to 0.15 ppm	2 elevated air pollution events, 1 low air pollution period	General population	Increase in reported eye and throat irritation, chest discomfort, shortness of breath, restricted activity and medical visits during high pollution events
●424 ●474	Xu et al., 1995 a, b	38 ppb increase	Daily	General population	A 38ppb increase in SO_2 associated with internal medicine and pediatric outpatient visits, and emergency room visits.
●429	Park et al., 2002	2.68 to 28.11 ppb	Unknown	Elementary school children	Associated with illness-related absences from school
***Epidemiology** – Case reports*					
◆272	Rabinovitch et al., 1989	"High"	Brief, accidental	Workers	Airway effects, reduced exercise tolerance
●021	Harkonen et al., 1983	"High"	Brief, accidental	Workers	Thoracic pain, coughing, conjunctival irritation, corneal erosion
●271	Galea, 1964	"High"	Brief, accidental	Workers	Dry irritable cough, dyspnea, copious amounts of mucous 10 d post-exposure
●269	Woodford et al., 1979	"High"	Brief, accidental	Workers	Burning and tearing eyes, rhinorrhea, cough, almost passing out
●270	Charan et al., 1979	"High"	Brief,	Workers	Irritation and soreness of eyes, nose

			accidental		and throat, tightness in chest, intense dyspnea, severe conjunctivitis and corneal burns

Cardiovascular System

Clinical					
◆071	Tunnicliffe et al. 2001	200 ppb	1 hr	Healthy and asthmatic	Differences in "total power" in healthy subjects
●032	Amdur et al., 1953	1 to 8 ppm	10 min	Healthy	No effect
Non-Clinical					
▲183	Fedde and Kuhlman, 1979	100 ppm 5000 ppm	1 hr	Chickens	No effect @ 100 ppm Increased heart rate @ 5000 ppm
◆211	Wang et al., 1996	5000 ppm	2 breaths	Rats	Decreased heart rate; no change in blood pressure (BP)
◆233	Callanan et al., 1974	100 to 400 ppb	1 to 3 min	Geese	Increased BP and heart rate
●241	Drew et al., 1983	50 ppm	6 hr/d, 5 d/wk, 6 wk	Rats	Decreased blood pressure in hypertension-resistant rats; increased BP in other rats
◆251	Langley-Evans et al., 1996	5 to 100 ppm	5 hr/d, 7-28 days	Rats	Decreased glutathione in the heart
◆163	Haider, 1985	10 ppm	1 hr/d, 30 days	Guinea pigs	Increased cholesterol, total lipids, phospholipids and decreased gangliosides in the heart
●147	Rana et al., 1979	500 ppm	4 min	Squirrels	Decreased lipid levels and increased moisture content in heart
●237	Balchum et al., 1960	1.8 to 148 ppm	30 to 40 min	Dogs	Low, uniform $^{35}SO_2$ concentration in heart muscle
Epidemiology					

◆423	Wong et al., 2002	4 ppb increase		General Population (Hong Kong and London, England)	Significant positive association between 4 ppb increase and daily admissions for cardiac diseases
●459	Sunyer et al., 2002	Range 1.9 – 8 ppb	Same-day	General population	Significant increase in daily numbers of all cardiovascular admissions except stroke and particularly ischemic heart disease
●387	Morris et al., 1995	0.05 ppm	Unknown	General Population (US cities)	Inconsistent results for association between an increase of 0.5 ppm SO_2 and hospital admissions for congestive heart failure Highest average SO_2 levels – New York Lowest average SO_2 levels – Los Angeles
Cardiovascular – Epidemiology*- No association observed*					
●002	Derriennic et al., 1989	19 to 25 ppb	Unreported	General population	No statistically significant correlation
●388	Ponka and Virtanen, 1996a	0.08 – 36 ppb range	Unknown	General population	No significant associations with hospital admissions for ischemic cardiac and cerebrovascular diseases. .
●441	Peters et al., 2000	Mean 0.007 ppm	Unknown	(Massachusetts)	No association between implanted cardioverter defibrillator discharges and SO_2 concentrations

Eye

Clinical					
▲096	Kulle et al., 1986	1 ppm	4 hr/d, 3 d/wk, 3 wk	Healthy adults	No adverse effects
●065	Coe and Douglas,	50 ppm	5 min	Healthy adults	No subjective effect reported

	1982				
●121	Douglas and Coe, 1987	3 to 60 ppm	15 s	Not reported	Threshold for tear production = 5 ppm
Non-Clinical					
▲159	Haider et al., 1981	10 ppm	1 hr/d, 21 d	Guinea pigs	Signs of eye irritation
Epidemiology					
◆011	Cohen et al., 1974	0.01 to 0.15 ppm	Unreported	General population	Eye irritation reported during both high and low pollution periods
●267	NIOSH, 1984	0.2 to 1.8 ppm	5 to 8 months	Workers	Eye irritation present; link to SO_2 exposure undetermined
●021	Harkonen et al., 1983	"high levels"	20-45 min	Workers	Conjunctival irritation, corneal erosion
●269	Woodford et al., 1979	"high concentrations"	15-20 min	Workers	Burning and tearing of eyes

Gastrointestinal System

Non-Clinical					
◆460	Meng et al., 2003	8.4±0.8, 21±1, 43±3 ppm	Unreported	Mice	Increased levels of lipid peroxidation in stomachs and intestines of male and female mice

General Biochemical Effects

Clinical					
◆055	Trenga et al., 1999	0.5 ppm	10 min	Asthmatic adults	No correlation between plasma antioxidant concentrations and sensitivity to SO_2
◆312	Kienast et al., 1994b	0.3 to 1.5 ppm	30 and 60 min	Unknown	No conclusive evidence as to the measured amount of ROI that is sufficient to induce clinically relevant pulmonary fibrosis
●112	Gunnison and	0.3, 1, 3, 6 ppm	up to 120 hr	Health smokers and non-	Positive correlation between plasma

	Palmes, 1974			smokers	S-sulfonate and atmospheric SO_2
●265	Grote and Thews, 1973	Unreported	Unreported	Adults, health status not reported	Amount of SO_2 dissolving in human blood increases with increasing blood O_2 and CO_2 and decreasing blood pH
Non-clinical					
◆236	Etlik et al., 1995	10 ppm	1 hr/d, 30 d	Guinea pigs	Increased methemoglobin, sulfhemoglobin, lipoperoxidation, osmotic fragility
●142	Matsumura, 1970	330 ppm	30 min	Guinea pigs	Hematoglutination in 5 of 10 exposed animals
●254	Lee and Danner, 1966	6-310 ppm	60 min	Guinea pigs	Increased hemoglobin @ all concs.; Increased inorganic sulphur in blood above 19 ppm
▲152	Lovati et al., 1996	5 and 10 ppm	15 days, continuous	Rats	Dose-dependent increase in plasma triglycerides and increase in HDL cholesterol (normal and hypertensive rats); decrease in plasma triglycerides and increase in HDL cholesterol (diabetic rats)
◆151	Jonek et al., 1976	Unreported	50 min	Rats	Highest concentration in blood 2 hr post-exposure
◆192	Baskurt, 1988	0.87 ppm	24 hr	Rats	Whole blood and packed cell viscosities decreased
◆225	Azoulay et al., 1980	2 ppm	1 to 49 d	Rats	No effects observed
●193	Gause and Barker, 1978	5 to 20 ppm	7 d	Rats	10% of inhaled SO_2 found in blood or plasma within 1st 30 min of exposure
●302	Baskurt et al., 1990	1 ppm		Rats	No significant effects on hemoglobin
◆212	Vanjonack and Johnson, 1972	40 ppm	0.5 to 24 hr	Mice	Decreased plasma thyroxine levels at 12 and 24 hr exposure; increased plasma glucocorticoids at 1 and 12 hr

●380	Meng et al., 2002	5 to 32 ppm		Mice	Increased frequencies of polychromatic erythrocyte micronuclei formation (MNPCE)
●222	Gunnison and Benton, 1971	23.5 ppm	14 to 62 hr	Rabbits	Increased plasma and serum S-sulfonate levels
▲183	Fedde and Kuhlman, 1978	up to 5000 ppm	60 min	Chickens	No change at 100 ppm; Decreased blood pH and O_2, increased blood CO_2 at 5000 ppm

Immunological System

Study ID	**Reference**	**SO_2 Concentration**	**Time**	**Species/Population Group**	**Effect**
Clinical					
◆035	Winterton et al., 2001	0.5 ppm	10 min	Asthmatic adults	Increased response to SO_2 is associated with the wild-type allele of THF-alpha promoter polymorphism
◆048	Anderson et al., 1977	5 ppm	4 hr	Healthy adults	50% decrease in nasal mucous flow; no difference in # of people developing colds
◆083	Sandstrom et al., 1989a	4, 8 ppm	20 min	Healthy adults	Dose-dependent increase in macrophage activity 24 post-exposure
●091	Sandstrom et al., 1989c	4, 5, 8, 11 ppm	20 min	Healthy adults	Increased macrophage activity 24 post-exposure; return to pre-exposure levels within 72 hr post-exposure
◆103	Koenig et al., 1987	0.75 ppm	10 min	Allergic adults, no asthma	Investigation of mechanism of action of SO_2-induced bronchoconstriction
●058	Sheppard et al., 1981a	0.5 or 1 ppm	10 min	Asthmatic adults	Investigation of mechanism of action of SO_2-induced bronchoconstriction
Non-clinical					
▲172	Azoulay-Depuis et	10 ppm	up to 3 wk	Mice	Increased mortality, decreased

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
	al., 1982				survival time in exposed, infected mice
◆207	Ukai, 1977	0.03 to 0.1 ppm	4 wk	Mice	Antibodies to virus developed more rapidly and increased # of goblet cells
◆238	Fairchild, 1977	6 ppm	7 d	Mice	Inhibition of influenza virus growth
●134	Trimpe et al., 1986	27 ppm	Not clear	Hamsters	No difference in bacterial clearance rates
▲259	Park et al., 2001	0.1 ppm	5 hr/d, 5 d	Guinea pigs	Enhanced ovalbumin-induced asthmatic reactions
◆133	Riedel et al., 1988	0.1 to 16.6 ppm	8 hr/d, 5 d	Guinea pigs	Increased ovalbumin-specific antibodies and bronchoalveolar fluid
●201	Gause and Rowlands, 1975	Unclear	Not reported	Human lymphocyte membranes	Dose-dependent spectral change
◆209	Watson and Brain, 1980	250 ppm	3 hr	Mice	Increased uptake of Fe in airway epithelium
●199	Okuyama et al., 1979	3.4 to 18.5 ppm	1 to 14 d	Chickens	Increased # of macrophages, lymphocytes, plasma cells and neutrophils
●146	Norris and Jackson, 1989	200 ppm	2 hr	Dogs	Increased airway permeability to plasma proteins and cell shedding
Epidemiology					
◆005	Boezen et al., 1999	0.3 to 22 ppm	3 months/yr, 3 yr	Adolescents	Children with bronchial hypersensitivity and high serum IgE levels were more susceptible to air pollution (not SO_2 specifically).

Kidney and Liver

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
Non-clinical					
▲152	Lovati et al., 1996	5 and 10 ppm	15 days	Rats	Increased liver triglycerides in normal rats; decreased liver weight and liver triglycerides in diabetic rats
◆163	Haider, 1985	10 ppm	1 hr/d, 30 d	Guinea pigs	Decreased phospholipids, cholesterol, and lipid peroxidation in liver and kidney
◆251	Langley-Evans et al., 1996	5 to 100 ppm	5 hr/d, 7-28 d	Rats	Decreased glutathione levels in liver and kidney; decreased glutathione reductase activity
●237	Balchum et al., 1960	1.8 to 148 ppm	30 to 40 min	Dogs	Second highest SO_2 conc. found in kidney; low conc. in liver.

Metabolic System

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
Non-clinical					
◆261	Johnson et al., 1972	40 ppm	4 to 11 d	Mice	Decreased metabolism as measured by O_2 consumption
◆381	Meng, 2003	20 ppm	6 hr /day for 7 days	Mice	Decreased activist of Se-dependent glutathione peroxidase in all organs of both sexes Significant decrease of catalase activity in livers from both sexes
◆251	Langley-Evans et al., 1996	5-100 ppm	5 hr/d, 7-28 d	Rats	Varied enzyme activity in lung, liver, heart, kidney

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
●273	Leung et al., 1985	Not reported	Single dose	Rats	Metabolite of SO_2 inhibits glutathione s-transferase in liver and lungs
Non-clinical – *Lipid metabolism*					
▲152	Lovati et al., 1996	5 or 10 ppm	15 d	Rats	Dose-dependent increase in plasma triglycerides, decreased HDL cholesterol in normal rats; decreased plasma and liver triglycerides, increased HDL cholesterol in diabetic rats
◆163	Haider, 1985	10 ppm	1 hr/d, 30 d	Guinea pigs	Changes in lipid metabolism varying by organ; decreased lipid peroxidation

Nervous System

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
Non-clinical, Behavioural					
▲214	Petruzzi et al., 1996	5, 12, 30 ppm	24 d	Mice	Changed behaviour after exposure
◆217	Fiore et al., 1998	5, 12, 30 ppm	14 d	Mice	Changed behaviour in adults after prenatal exposure
Non-clinical, Biochemical					
▲159	Haider et al., 1981	10 ppm	1 hr/d, 21 d	Guinea pigs	Decreased total lipids and free fatty acids; lipid content and enzyme activity vary depending on brain area
◆249	Haider et al., 1982	10 ppm	1 hr/d, 30 d	Rats	Lipid content and enzyme activity vary depending on brain area
Non-clinical, Functional					
▲197	Barthelmy et al., 1988	0.5, 5 ppm	45 min	Rabbits	

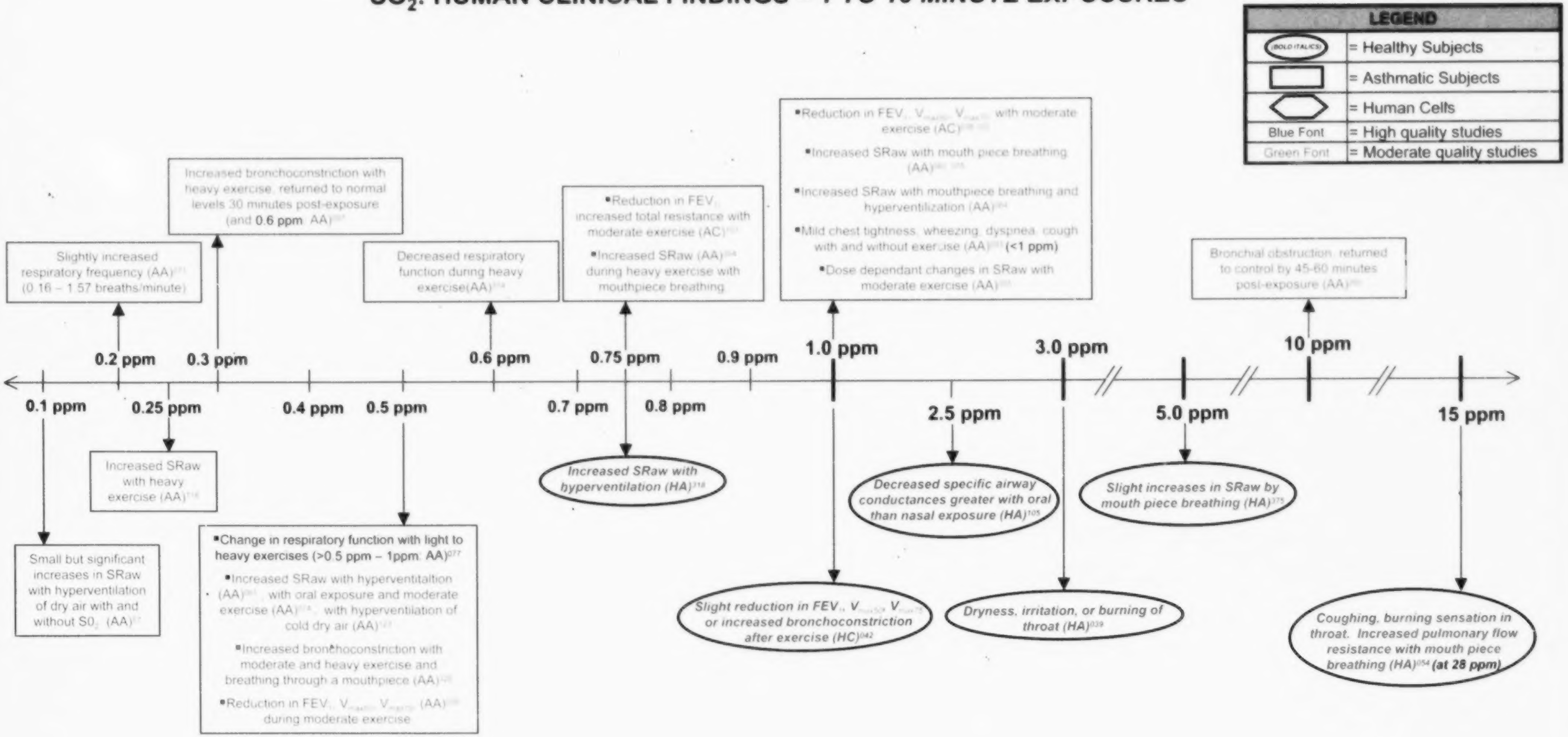
◆153	Karpas and Widdicombe, 1983	20000 ppm	single dose	Ferrets	Investigation of the reflexive nature of SO_2-induced bronchoconstriction
◆200	Matsumoto et al., 1997	200 ppm	Unknown	Rabbits	
◆211	Wang et al., 2001	5000 ppm	2 breaths	Rats	
●237	Balchum et al., 1960	1.8-148 ppm	30-40 min	Dogs	
◆239	Davies et al., 1978b	150 ppm 300 ppm	12x 3 hr 3 hr	Rabbits	
◆244	Davenport et al., 1984	200-400 ppm	15-20 min	Rabbits	
●069	Nadel et al., 1965	Unknown	Unknown	Cats	
●141	Mortola et al., 1985	300-350 ppm	10-15 min	Rabbits	
●161	Hanacek et al., 1991	200-300 ppm	10-20 min	Rabbits	
●167	Cho et al., 1968	100-10000 ppm	0.1-6 min	Dogs	
●194	Citterio et al., 1985a	300-350 ppm	Unknown	Rabbits	
●195	Citterio et al., 1985b	300-350 ppm	15-20 min	Rabbits	
●234	Davies et al., 1978a	200 ppm	1-5x 10 min	Rabbits	

Reproductive System

Study ID	Reference	SO_2 Concentration	Time	Species/Population Group	Effect
Non-clinical					
◆203	Singh, 1982	32, 65, 125, 250 ppm	Gestational days 7 to 17	Mice	No effect on # of dead or reabsorbed fetuses; no teratological effects; decreased pup weight at 65 and 125 ppm
◆140	Murray et al., 1979	25 ppm 70 ppm	Gestational days 6 to 15 Gestational days 6 to 18	Mice Rabbits	Decreased mean fetal body weight; delayed ossification of sternebrae and occipital bone Minor skeletal variations
▲214	Petruzzi et al., 1996	5, 12, 30 ppm	From 9 days pre-pregnancy to gestational	Mice	No changes observed in reproductive performance of neurobehavioural development of offspring

			day 12-14		
◆217	Fiore et al., 1998	5, 12, 30 ppm	Gestational days 1 to 14	Mice	Enhancement in body sniffing and self-grooming behaviour; increased other social behaviour; decreased tail rattling, freezing and defensive behaviours
Epidemiology					
●003	Dolk et al., 2000	Unreported; based on distance from point source	Unreported	General population	No evidence of adverse birth outcomes with respect to distance from cokeworks (point source of SO_2)

RESPIRATORY EFFECTS ASSOCIATED WITH SHORT-TERM EXPOSURE TO SO_2: HUMAN CLINICAL FINDINGS – *1 TO 10 MINUTE EXPOSURES*



LEGEND	
(BOLD ITALICS)	= Healthy Subjects
▭	= Asthmatic Subjects
⬡	= Human Cells
Blue Font	= High quality studies
Green Font	= Moderate quality studies

0.1 ppm: Small but significant increases in SRaw with hyperventilation of dry air with and without SO_2 (AA)

0.2 ppm: Slightly increased respiratory frequency (AA) (0.16 – 1.57 breaths/minute)

0.25 ppm: Increased SRaw with heavy exercise (AA)

0.3 ppm: Increased bronchoconstriction with heavy exercise, returned to normal levels 30 minutes post-exposure (and **0.6 ppm**: AA)

0.5 ppm:

- **Change in respiratory function with light to heavy exercises (>0.5 ppm – 1ppm: AA)[077]**
- Increased SRaw with hyperventitaltion (AA), with oral exposure and moderate exercise (AA), with hyperventilation of cold dry air (AA)
- Increased bronchoconstriction with moderate and heavy exercise and breathing through a mouthpiece (AA)
- Reduction in FEV_1, V_{max50}, V_{max75} (AA) during moderate exercise

0.6 ppm: Decreased respiratory function during heavy exercise(AA)

0.75 ppm:

- Reduction in FEV_1, increased total resistance with moderate exercise (AC)
- Increased SRaw (AA) during heavy exercise with mouthpiece breathing
- ***Increased SRaw with hyperventilation (HA)[318]***

1.0 ppm:

- Reduction in FEV_1, V_{max50}, V_{max75} with moderate exercise (AC)
- Increased SRaw with mouth piece breathing (AA)
- Increased SRaw with mouthpiece breathing and hyperventilization (AA)
- Mild chest tightness, wheezing, dyspnea, cough with and without exercise (AA) **(<1 ppm)**
- Dose dependant changes in SRaw with moderate exercise (AA)
- ***Slight reduction in FEV_1, V_{max50}, V_{max75} or increased bronchoconstriction after exercise (HC)[042]***

2.5 ppm: ***Decreased specific airway conductances greater with oral than nasal exposure (HA)[105]***

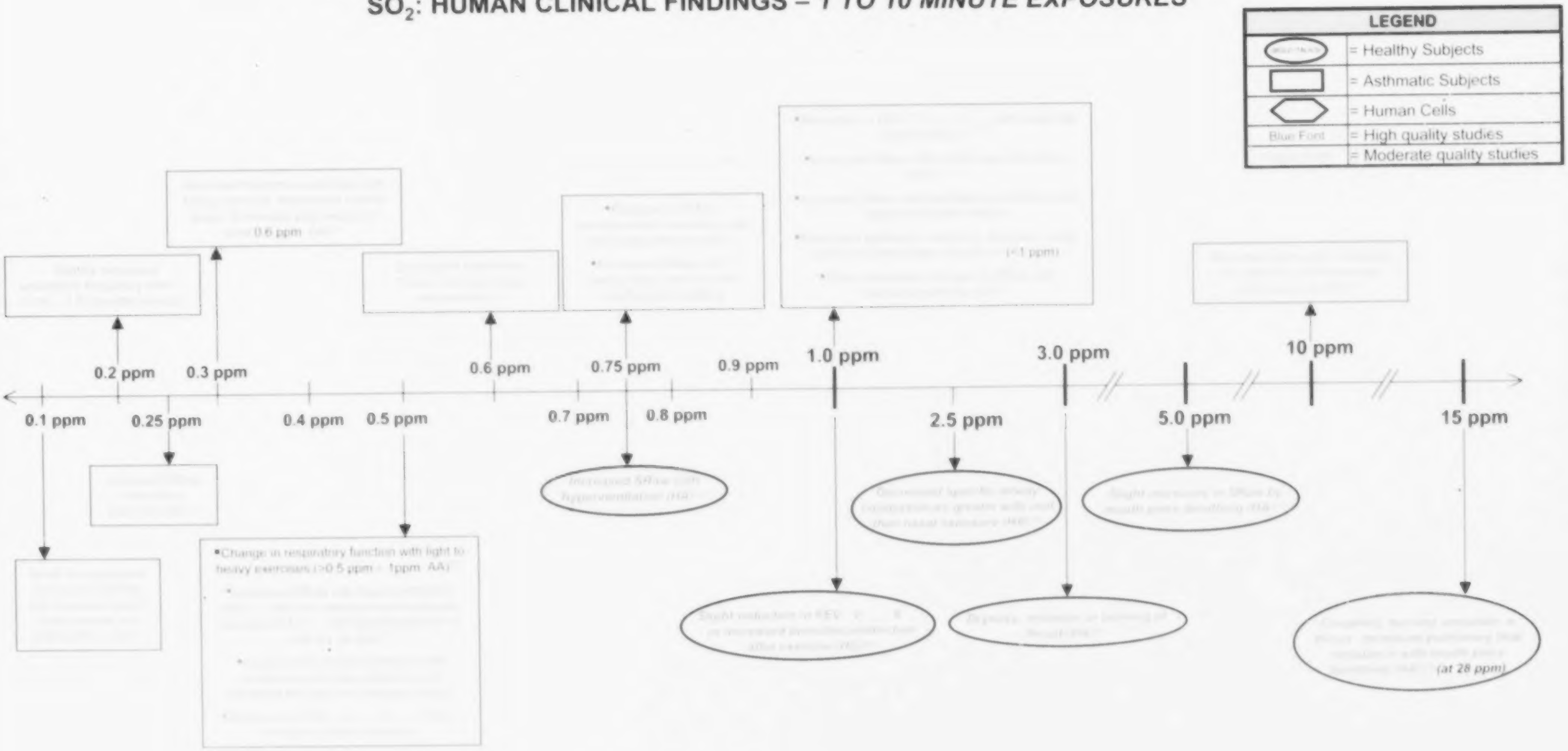
3.0 ppm: ***Dryness, irritation, or burning of throat (HA)[039]***

5.0 ppm: ***Slight increases in SRaw by mouth piece breathing (HA)[375]***

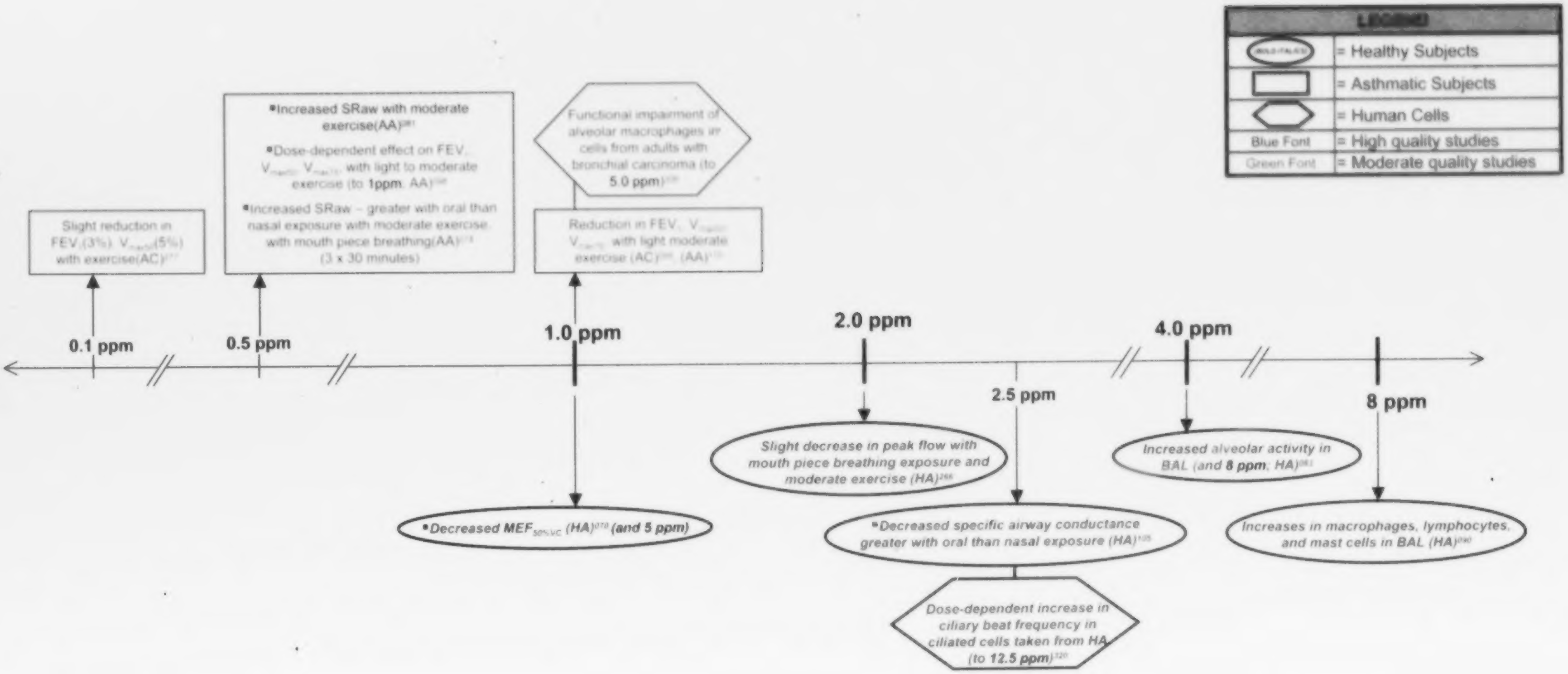
10 ppm: Bronchial obstruction, returned to control by 45-60 minutes post-exposure (AA)

15 ppm: ***Coughing, burning sensation in throat. Increased pulmonary flow resistance with mouth piece breathing (HA)[054] (at 28 ppm)***

RESPIRATORY EFFECTS ASSOCIATED WITH SHORT-TERM EXPOSURE TO SO_2: HUMAN CLINICAL FINDINGS – *1 TO 10 MINUTE EXPOSURES*


LEGEND
= Healthy Subjects
= Asthmatic Subjects
= Human Cells
Blue Font = High quality studies
= Moderate quality studies
0.1 ppm
0.2 ppm
0.25 ppm
0.3 ppm
0.4 ppm
0.5 ppm
0.6 ppm
0.7 ppm
0.75 ppm
0.8 ppm
0.9 ppm
1.0 ppm
2.5 ppm
3.0 ppm
5.0 ppm
10 ppm
15 ppm
0.6 ppm
(<1 ppm)
•Change in respiratory function with light to heavy exercises (>0.5 ppm – 1ppm, AA)
Increased SRaw with hyperventilation (HA)
Slight increases in SRaw by mouth piece breathing (HA)
Dryness, irritation or burning of throat (HA)
(at 28 ppm)

RESPIRATORY EFFECTS ASSOCIATED WITH SHORT-TERM EXPOSURE TO SO_2: HUMAN CLINICAL FINDINGS – *11 TO 30 MINUTE EXPOSURES*



LEGEND	
⬭	= Healthy Subjects
▭	= Asthmatic Subjects
⬡	= Human Cells
Blue Font	= High quality studies
Green Font	= Moderate quality studies

0.1 ppm

Slight reduction in FEV$_1$(3%), V_{max50}(5%) with exercise(AC)[177]

0.5 ppm

- Increased SRaw with moderate exercise(AA)[281]
- Dose-dependent effect on FEV$_1$, $V_{max(50)}$, $V_{max(75)}$ with light to moderate exercise (to **1ppm**, AA)[128]
- Increased SRaw – greater with oral than nasal exposure with moderate exercise, with mouth piece breathing(AA)[173] (3 x 30 minutes)

1.0 ppm

Reduction in FEV$_1$, $V_{max(50)}$, $V_{max(75)}$ with light moderate exercise (AC)[illegible], (AA)[illegible]

Functional impairment of alveolar macrophages in cells from adults with bronchial carcinoma (to **5.0 ppm**)[illegible]

- *Decreased **MEF$_{50\%VC}$** (HA)[070] **(and 5 ppm)***

2.0 ppm

Slight decrease in peak flow with mouth piece breathing exposure and moderate exercise (HA)[266]

2.5 ppm

- *Decreased specific airway conductance greater with oral than nasal exposure (HA)[105]*

*Dose-dependent increase in ciliary beat frequency in ciliated cells taken from HA (to **12.5 ppm**)[120]*

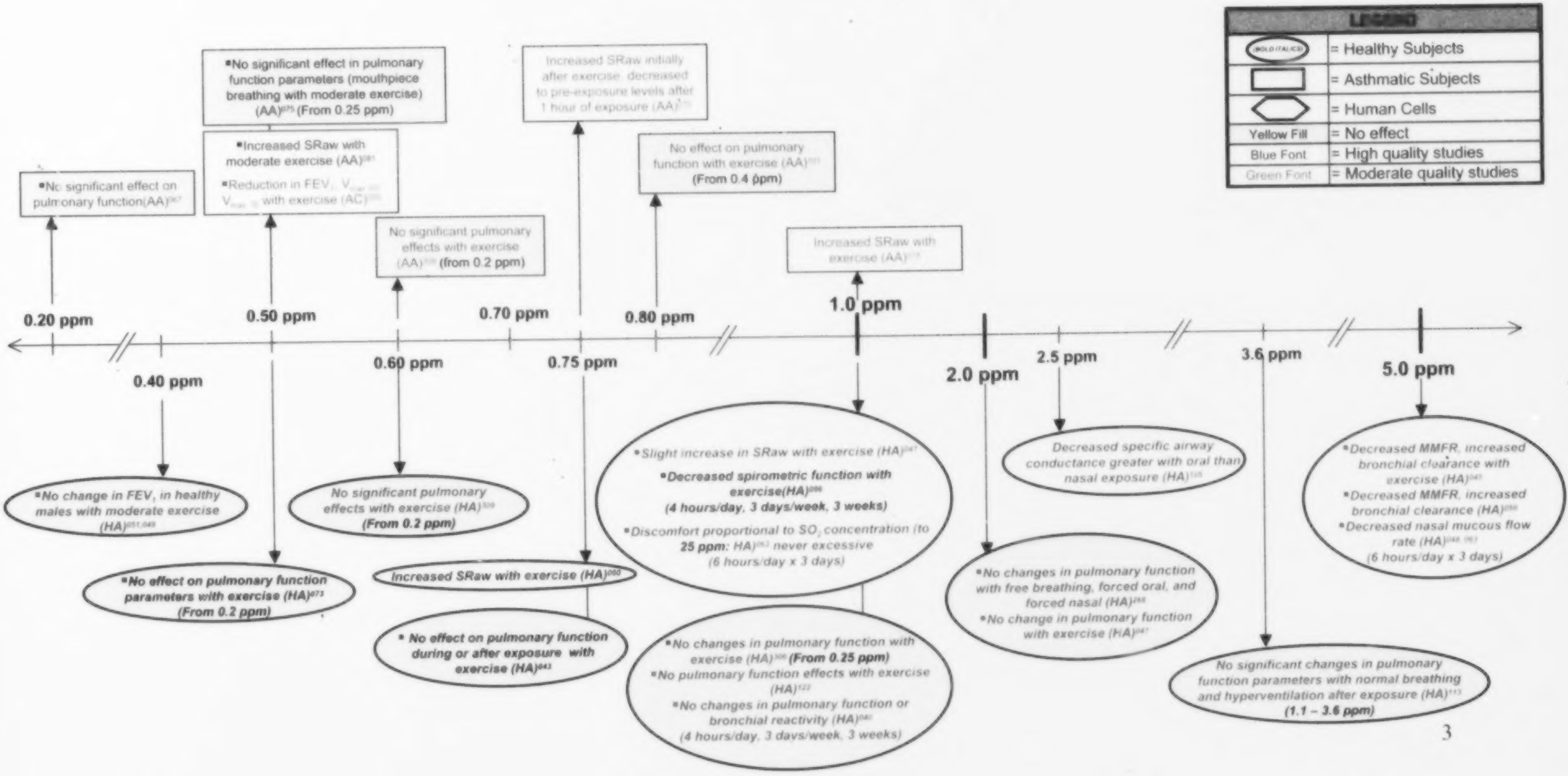
4.0 ppm

*Increased alveolar activity in BAL (and **8 ppm**; HA)[061]*

8 ppm

Increases in macrophages, lymphocytes, and mast cells in BAL (HA)[090]

RESPIRATORY EFFECTS ASSOCIATED WITH SHORT-TERM EXPOSURE TO SO_2: HUMAN CLINICAL FINDINGS – *30 MINUTE TO 4 HOUR EXPOSURE AND 3 DAYS TO 3 WEEK EXPOSURE*



LEGEND	
BOLD ITALICS ⬭	= Healthy Subjects
▭	= Asthmatic Subjects
⬡	= Human Cells
Yellow Fill	= No effect
Blue Font	= High quality studies
Green Font	= Moderate quality studies

0.20 ppm

- No significant effect on pulmonary function(AA)[067]

0.40 ppm

- ***No change in FEV$_1$ in healthy males with moderate exercise (HA)[051,049]***

0.50 ppm

- No significant effect in pulmonary function parameters (mouthpiece breathing with moderate exercise) (AA)[075] (From 0.25 ppm)
- Increased SRaw with moderate exercise (AA)[081]
- Reduction in FEV$_1$, $V_{max\ 50}$, $V_{max\ 75}$ with exercise (AC)[illegible]
- ***No effect on pulmonary function parameters with exercise (HA)[073] (From 0.2 ppm)***

0.60 ppm

No significant pulmonary effects with exercise (AA)[309] (from 0.2 ppm)

No significant pulmonary effects with exercise (HA)[309] (From 0.2 ppm)

0.70 ppm

0.75 ppm

Increased SRaw initially after exercise, decreased to pre-exposure levels after 1 hour of exposure (AA)[illegible]

Increased SRaw with exercise (HA)[060]

- ***No effect on pulmonary function during or after exposure with exercise (HA)[043]***

0.80 ppm

No effect on pulmonary function with exercise (AA)[101] (From 0.4 ppm)

1.0 ppm

Increased SRaw with exercise (AA)[illegible]

- *Slight increase in SRaw with exercise (HA)[047]*
- ***Decreased spirometric function with exercise(HA)[096]***
(4 hours/day, 3 days/week, 3 weeks)
- *Discomfort proportional to SO_2 concentration (to* ***25 ppm:*** *HA)[063] never excessive*
(6 hours/day x 3 days)

- *No changes in pulmonary function with exercise (HA)[306]* ***(From 0.25 ppm)***
- *No pulmonary function effects with exercise (HA)[122]*
- *No changes in pulmonary function or bronchial reactivity (HA)[040]*
(4 hours/day, 3 days/week, 3 weeks)

2.0 ppm

- *No changes in pulmonary function with free breathing, forced oral, and forced nasal (HA)[268]*
- *No change in pulmonary function with exercise (HA)[047]*

2.5 ppm

Decreased specific airway conductance greater with oral than nasal exposure (HA)[105]

3.6 ppm

No significant changes in pulmonary function parameters with normal breathing and hyperventilation after exposure (HA)[113] ***(1.1 – 3.6 ppm)***

5.0 ppm

- *Decreased MMFR, increased bronchial clearance with exercise (HA)[043]*
- *Decreased MMFR, increased bronchial clearance (HA)[059]*
- *Decreased nasal mucous flow rate (HA)[048, 063]*
(6 hours/day x 3 days)

RESPIRATORY EFFECTS ASSOCIATED WITH SHORT-TERM EXPOSURE TO SO_2: ANIMAL TOXICOLOGY STUDIES – *UP TO 2 HOUR EXPOSURES*

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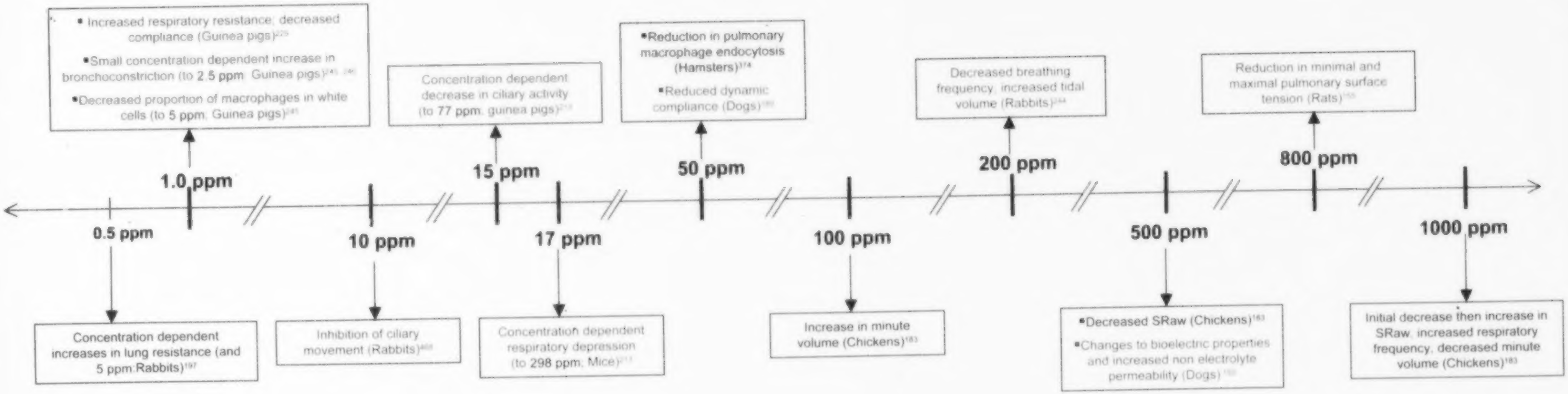

0.5 ppm
Concentration dependent increases in lung resistance (and 5 ppm:Rabbits)[197]

1.0 ppm
- Increased respiratory resistance; decreased compliance (Guinea pigs)[225]
- Small concentration dependent increase in bronchoconstriction (to 2.5 ppm: Guinea pigs)[245, 246]
- Decreased proportion of macrophages in white cells (to 5 ppm: Guinea pigs)[241]

10 ppm
Inhibition of ciliary movement (Rabbits)[408]

15 ppm
Concentration dependent decrease in ciliary activity (to 77 ppm: guinea pigs)[213]

17 ppm
Concentration dependent respiratory depression (to 298 ppm: Mice)[211]

50 ppm
- Reduction in pulmonary macrophage endocytosis (Hamsters)[174]
- Reduced dynamic compliance (Dogs)[180]

100 ppm
Increase in minute volume (Chickens)[183]

200 ppm
Decreased breathing frequency, increased tidal volume (Rabbits)[244]

500 ppm
- Decreased SRaw (Chickens)[183]
- Changes to bioelectric properties and increased non electrolyte permeability (Dogs)[180]

800 ppm
Reduction in minimal and maximal pulmonary surface tension (Rats)[155]

1000 ppm
Initial decrease then increase in SRaw, increased respiratory frequency, decreased minute volume (Chickens)[183]

RESPIRATORY EFFECTS ASSOCIATED WITH SHORT-TERM EXPOSURE TO SO_2: ANIMAL TOXICOLOGY STUDIES – *2 HOUR TO 1 DAY EXPOSURES*

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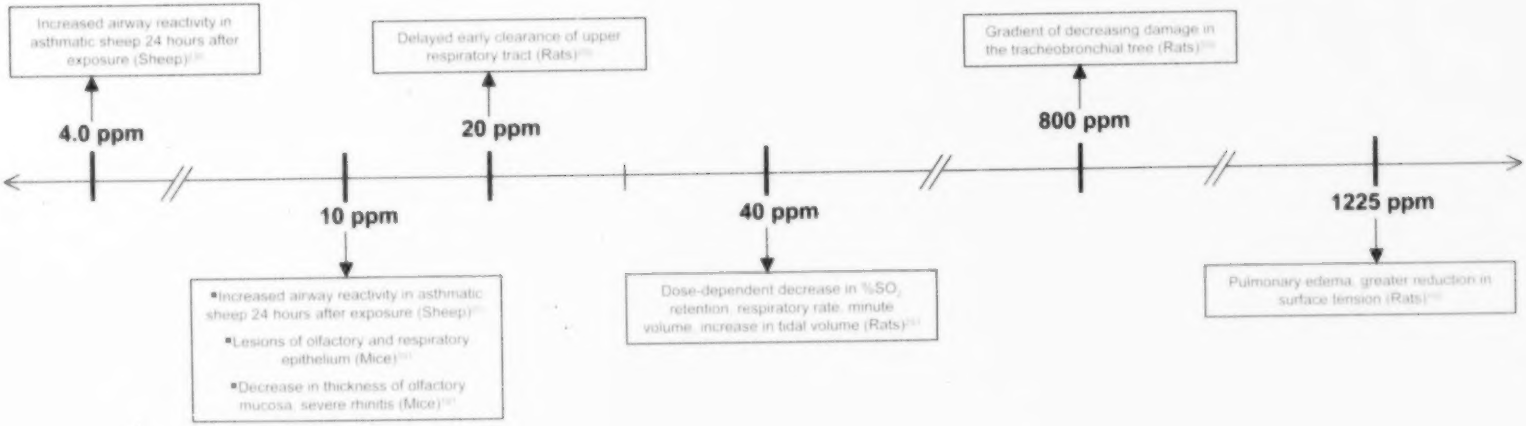

4.0 ppm

Increased airway reactivity in asthmatic sheep 24 hours after exposure (Sheep)

10 ppm

- Increased airway reactivity in asthmatic sheep 24 hours after exposure (Sheep)
- Lesions of olfactory and respiratory epithelium (Mice)
- Decrease in thickness of olfactory mucosa, severe rhinitis (Mice)

20 ppm

Delayed early clearance of upper respiratory tract (Rats)

40 ppm

Dose-dependent decrease in %SO_2 retention, respiratory rate, minute volume, increase in tidal volume (Rats)

800 ppm

Gradient of decreasing damage in the tracheobronchial tree (Rats)

1225 ppm

Pulmonary edema, greater reduction in surface tension (Rats)

RESPIRATORY EFFECTS ASSOCIATED WITH SHORT-TERM EXPOSURE TO SO_2: ANIMAL TOXICOLOGY STUDIES – *1 DAY TO 7 DAY EXPOSURES*

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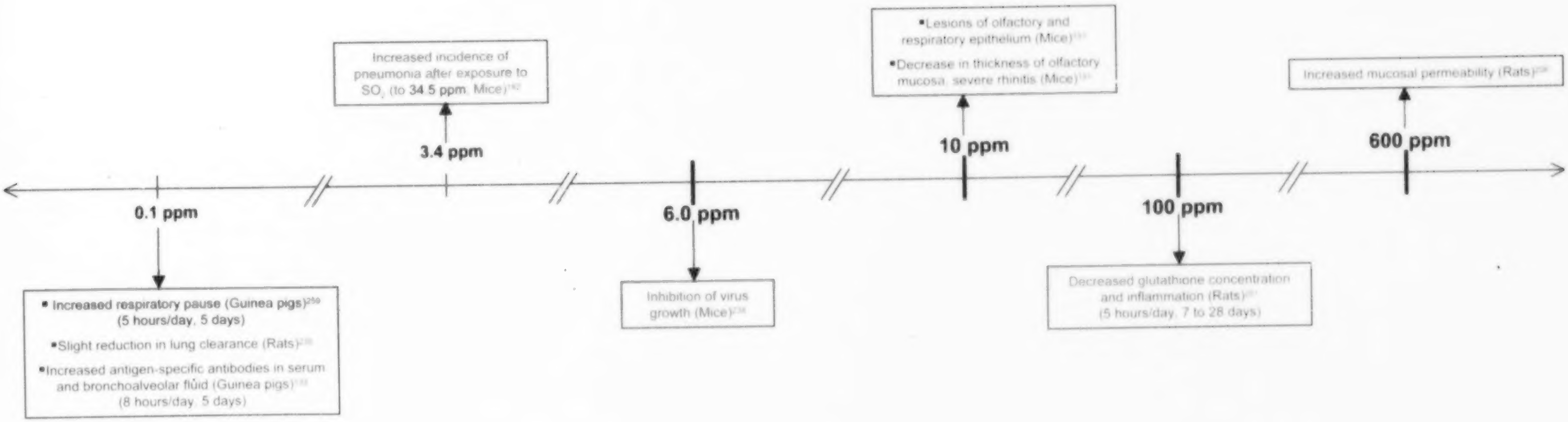
Green Font	= Moderate quality studies



- **Increased respiratory pause (Guinea pigs)[250] (5 hours/day, 5 days)**
- Slight reduction in lung clearance (Rats)[illegible]
- Increased antigen-specific antibodies in serum and bronchoalveolar fluid (Guinea pigs)[illegible] (8 hours/day, 5 days)

Increased incidence of pneumonia after exposure to SO_2 (to 34.5 ppm, Mice)[182]

Inhibition of virus growth (Mice)[238]

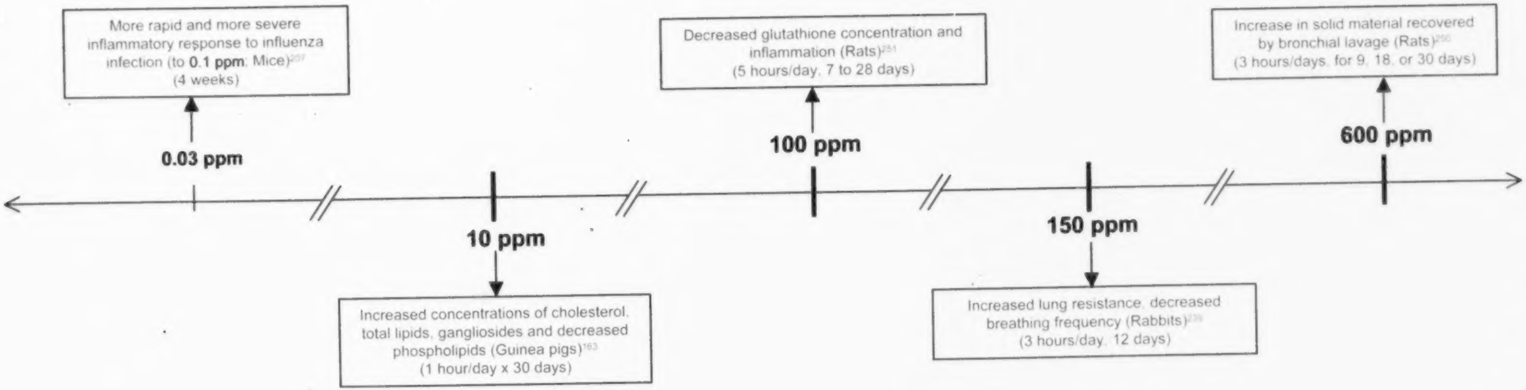
- Lesions of olfactory and respiratory epithelium (Mice)[illegible]
- Decrease in thickness of olfactory mucosa, severe rhinitis (Mice)[illegible]

Decreased glutathione concentration and inflammation (Rats)[illegible] (5 hours/day, 7 to 28 days)

Increased mucosal permeability (Rats)[illegible]

RESPIRATORY EFFECTS ASSOCIATED WITH SHORT-TERM EXPOSURE TO SO_2: ANIMAL TOXICOLOGY STUDIES – *EXPOSURES GREATER THAN 7 DAYS AND UP TO 30 DAYS*

[illegible]	
Blue Font	= High quality studies
Green Font	= Moderate quality studies



0.03 ppm

More rapid and more severe inflammatory response to influenza infection (to **0.1 ppm**: Mice)[207]
(4 weeks)

10 ppm

Increased concentrations of cholesterol, total lipids, gangliosides and decreased phospholipids (Guinea pigs)[163]
(1 hour/day x 30 days)

100 ppm

Decreased glutathione concentration and inflammation (Rats)[251]
(5 hours/day, 7 to 28 days)

150 ppm

Increased lung resistance, decreased breathing frequency (Rabbits)[239]
(3 hours/day, 12 days)

600 ppm

Increase in solid material recovered by bronchial lavage (Rats)[250]
(3 hours/days, for 9, 18, or 30 days)

APPENDIX 6

DETAILED DOCUMENT REVIEW

Author: Piirila, P. L. Nordman, H. Korhonen, O. S. Winblad, I.	**Internal ID:** **001**

Title: A thirteen-year follow-up of respiratory effects of acute exposure to sulphur dioxide

Year:1996

Abstract: OBJECTIVES: In 1977, nine men were accidentally exposed to sulphur dioxide in an explosion in a pyrite mine. The lung function of seven men was followed after the accident. A four-year follow-up has been published previously. The greatest decrease in forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1.0), and maximal midexpiratory flow (MMEF) was observed one week after the accident, after which all these parameters improved without reaching the preaccident level. Reversible bronchial obstruction was still present in three patients, and a positive reaction in the histamine challenge test was found for four. In the present paper, the lung function follow-up 13 years after the accident is reported for six men. METHODS: The patients' clinical condition, chest X-ray, spirometry, and histamine challenge test were studied 13 years after the incident. RESULTS: Spirometry was normal in one worker, two displayed obstruction, and three had a combined obstructive and restrictive, mainly obstructive, ventilatory impairment. In the histamine challenge test, four patients showed bronchial hyperreactivity, one with a nearly significant reaction. Because of bronchial obstruction one patient could not perform the challenge test. CONCLUSIONS: This 13-year follow-up showed that acute inflammatory obstruction caused by exposure to sulphur dioxide left, as sequelae, obstructive impairment of ventilatory function and permanent bronchial hyperreactivity. The clinical picture displayed by these patients was named the "reactive airways dysfunction syndrome" (RADS) in 1985. Four of the patients also showed symptoms of chronic bronchitis.

Objective: In 1977, nine men were accidentally exposed to sulphur dioxide in an explosion in a pyrite mine. The lung function of seven men was followed after the accident. A four-year follow-up has been published previously. The greatest decrease in forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1.0), and maximal midexpiratory flow (MMEF) was observed one week after the accident, after which all these parameters improved without reaching the preaccident level. Reversible bronchial obstruction was still present in three patients, and a positive reaction in the histamine challenge test was found for four. In the present paper, the lung function follow-up 13 years after the accident is reported for six men. The patients' clinical condition, chest X-ray, spirometry, and histamine challenge test were studied 13 years after the incident.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Acute single	**20-25 min**	**male**	**35-48 (?)**	**6**	**4 heavy smokers**		

Observations:

The greatest decrease in forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1.0), and maximal midexpiratory flow (MMEF) was observed one week after the accident, after which all these parameters improved without reaching the preaccident level. Reversible bronchial obstruction was still present in three patients, and a positive reaction in the histamine challenge test was found for four. In the present paper, the lung function follow-up 13 years after the accident is reported for six men. Spirometry was normal in one worker, two displayed obstruction, and three had a combined obstructive and restrictive, mainly obstructive, ventilatory impairment. In the histamine challenge test, four patients showed bronchial hyperreactivity, one with a nearly significant reaction. Because of bronchial obstruction one patient could not perform the challenge test.

*Statistically significant

Author's Conclusions:

This 13-year follow-up showed that acute inflammatory obstruction caused by exposure to sulphur dioxide left, as sequelae, obstructive impairment of ventilatory function and permanent bronchial hyperreactivity. The clinical picture displayed by these patients was named the "reactive airways dysfunction syndrome" (RADS) in 1985. Four of the patients also showed symptoms of chronic bronchitis.

Review – Assessment:

A. Subjects:	✎ Is the number of subjects sufficient to produce statistically significant results?- ✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study?+ ✎ Are the subjects a representative sample of the population to which extrapolations will be made?- ✎ Were identical 'criteria for exclusion' applied to both cases and controls?NA ✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?-
B. Exposure conditions:	✎ Was the exposure concentration measured?- ✎ Is the exposure duration known?+
C. Equipment:	✎ Was a full description of the monitoring devices provided?- ✎ What was the sensitivity of the monitoring devices employed?+
D. Procedural:	✎ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval?+ ✎ Were appropriate controls recruited?- ✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc)- ✎ Was the timing and length of follow-up appropriate?+ ✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)+
E. Data collection:	✎ Was the assessment identical for cases and controls? NA ✎ Were appropriate endpoints examined?+ ✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom)+ ✎ Were accepted assessment methods employed?+ ✎ Were assessment measures sufficiently sensitive+ ✎ Was the nature and severity of symptoms graded against an explicitly defined scale?+ ✎ Did qualified personnel conduct the exams?+ ✎ Was the timing and frequency of assessment logical?+ ✎ Was raw data provided to permit the reader to arrive at his/her own conclusions?+ ✎ Was individual data reported for each subject?+ ✎ Are all withdrawals listed, with the reason for withdrawal?+

F. Data analysis:	✎ Are the statistical methods used appropriate?+ ✎ Are confidence intervals reported?- ✎ Did the investigator appropriately interpret the statistics?+ ✎ Are the observed trends statistically significant?+
G. Interpretations:	✎ Was the original objective addressed?+ ✎ Are the results novel?+ ✎ Was the design of the study appropriate to investigate the primary objective?+ ✎ Were assumptions reasonable?+ ✎ Are the methods reproducible?+ ✎ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)?- ✎ Do the conclusions make biological sense?+ ✎ Are the extrapolations reasonable?+ ✎ Is the paper in a peer-reviewed journal?+ ✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?-

Review - Summary:

Discussion of findings:

Reviewer's summary of study findings and interpretation of the toxicological significance and clinical relevance of these findings. This report shows that the bronchial hyperreactivity seen in reactive airways dysfunction syndrome can develop into a permanent pathological state. Bronchial hyperreactivity persists in persons accidentally exposed to sulphur dioxide even after 13 years. However, while the subjects' age, symptoms, anthropometric and smoking data were recorded, they were not controlled for within the experiment. The lack of a measurement of physical activity and true quantification of what characteristics could be due to smoking and those caused by the sulphur dioxide do render the results somewhat suspect. However, because they involved only a few young, healthy, working-class men, extrapolation of the data to the general population would be questionable.

Confidence index:

The study investigated only six of the original seven men included in the 1980. Absence of the seventh man from the investigation was not explained. A sample size of six, while representative of the original nine men, cannot produce statistically significant results. One member of the investigative team is affiliated with the mine where the explosion occurred, raising questions about possible bias incorporated into the findings. Also, as reported by the authors, the methods of monitoring lung function had changed to some extent since the subjects were last examined in 1980, which may limit the comparison with previous data. The men were also not studied with histamine until 4 years after the incident, which introduces some uncertainty when comparing to preaccident data.

Three of the men were still working in the same workplace and one patient retired five years prior to this follow-up. Exposure to SO_2 may have occurred over these years. Information regarding additional exposures over the last thirteen years is not provided. Two of the patients had been reassigned to other work tasks, but no details are provided.

Review – Confidence index ranking:

Moderate

Author: Derriennic, R., Richardson, S., Mollie, A. and Lellouch, J.	**Internal ID:** 002

Title:
Short-term Effects of Sulphur Dioxide Pollution on Mortality in Two French Cities

Year: 1989

Abstract:
To assess the short-term effects of sulphur dioxide (SO_2) atmospheric pollution on mortality a study was conducted in two large French cities, using daily measures of pollution and mortality. A statistically significant association between daily SO_2 pollution and respiratory deaths up to 10 days later was shown in both cities for both male and female populations together in the age group 65 years and over. No coherent results were found between SO_2 pollution and cardiovascular deaths, or between suspended particulates and either respiratory or cardiovascular deaths. Particular emphasis was put on the use of correct statistical procedures using time series analysis and on the comparison of the part played by SO_2 or suspended particulates on mortality.

Objective:
To assess the short-term effects of sulphur dioxide pollution on mortality in two French cities. Also, to determine at what level of pollution that health effects are measurable.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Lyons: approximately 65.0 µg/m^3; Marseilles: approximately 50.7 µg/m^3.	-	Male and female	All ages	Two cities	-	-	-

Observations:

Fluctuations in total number of deaths and in respiratory deaths differed significantly from a Poisson distribution, suggesting that observed variations could not be fully explained by sampling fluctuations. Highly significant negative correlations between SO_2 levels and temperature. Each city exhibited different results between SO_2 and cardiovascular diseases. Marseilles exhibited asymmetric cross-correlation plots while Lyons did not show this relationship. Cross-correlation plots between SO_2 and mortality for all causes was asymmetric in Marseilles but not in Lyons. However, there was an asymmetry about zero for the cross-correlation plots between the number of respiratory deaths and SO_2 levels for the two cities. Results indicated positive and statistically significant cross-correlation coefficients between SO_2 and respiratory deaths up to 10 days later.

*Statistically significant

Author's Conclusions:

Results showed an effect of atmospheric pollution from SO_2 on mortality from respiratory causes in people aged 65 and over. The effect was observed independently in two major French urban areas that were similar in size but with different climatic conditions.

"When investigating the effect of SO_2 on daily morality, an asymmetric correlogram was only observed for people over 65 years of age who belong to a group that can be reasonably supposed to be more sensitive to environmental exposure. The asymmetry of the correlogram indicates that SO_2 measured on a given day is correlated to mortality on the following days but not to previous days. This provides an argument in favour of a causal relationship between SO_2 pollution and

mortality."

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+/-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? N/A Were identical 'criteria for exclusion' applied to both cases and controls? N/A Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (-) Is the exposure duration known?
C. Equipment:	(+) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? N/A Were appropriate controls recruited? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	N/A Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? N/A Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? N/A Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? N/A Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?

G. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The study examined the effect of sulphur dioxide on mortality in two French cities. Thorough statistical investigation revealed a significant association between daily SO_2 pollution and respiratory deaths up to 10 days later in an age group of 65 years and over. The study recognized confounding factors such as temperature and gender, and found that there was no significant changes to the results caused by these factors. This study builds on previous research focussed on the long term effects of air pollution on respiratory symptoms and pulmonary function by examining individual pollutants. Findings might be significant from a public health perspective if a particularly susceptible population subgroup was identified. To make valid comparisons between study results and other geographies, similar statistical analysis techniques should be used.

Confidence index:

The study made use of data collected by the French National Survey, conducted from 1974 to 1976 in 28 areas. The span of the study coupled with the number of subjects involved enables results to be considered highly statistically significant. While pollution levels in the cities may have significantly changed in the ensuing years, the statistical analysis produced valid results. Cause of death was taken from death certificates, which may have led to misclassification of cause of death. Furthermore, no information was given on the general health of the patients that died of respiratory disease. Observed fluctuations in mortality were compared with results obtained from a Poisson distribution, to determine if cause of death extended beyond sampling fluctuations. This enabled comparison of actual mortality with expected results. Because this study was not conducted in a controlled environment, exact exposure of each subject to pollutants could not be recorded. Furthermore, it is difficult to attribute increases in mortality or morbidity to a single air pollutant. However, the statistics employed by the researchers attempted to minimize possible bias by accounting for seasonality, temperature and gender.

Review – Confidence index ranking:

Low

Author: Dolk H, Pattenden S, Vrijheid M, Thakrar B, Armstrong B.	**Internal ID:** 003

Title: Perinatal and infant mortality and low birth weight among residents near cokeworks in Great Britain.

Year:2000

Abstract: With growing evidence of the adverse health effects of air pollution--especially fine particulates--investigators must concentrate on the fetus, neonate, and infant as potentially vulnerable groups. Cokeworks are a major source of smoke and sulphur dioxide. In the current study, the authors investigated whether populations residing near cokeworks had a higher risk of adverse perinatal and infant outcomes. Zones of 7.5-km radius around 22 cokeworks in Great Britain were studied, within which the authors assumed that exposure declined from highest levels within 2 km to background levels. Routinely recorded birth and death data for Great Britain during the period 1981-1992 were analyzed. Each individual record had a postcode that referred to a small geographical area of typically 15-17 addresses. The authors calculated expected numbers on the basis of regional rates, stratified by year, sex, and a small-area socioeconomic deprivation score. For all cokeworks combined, the observed/expected ratio (95% confidence intervals within parentheses) within 2 km of cokeworks was 1.00 (0.95, 1.06) for low-birth-weight (i.e., < 2,500 g) infants; 0.94 (0.78, 1.12) for still births; 0.95 (0.83, 1.09) for infant mortality; 0.86 (0.72, 1.03) for neonatal mortality; 1.10 (0.90, 1.33) for postneonatal mortality; 0.79 (0.30, 1.46) for respiratory postneonatal mortality; and 1.07 (0.77, 1.43) for postneonatal Sudden Infant Death Syndrome. Respiratory postneonatal mortality was low throughout the entire 0-7.5-km study area (observed/expected = 0.74 [0.61, 0.88]). There was no statistically significant decline in risk with distance from cokeworks for any of the outcomes studied. The authors concluded that there was no evidence of an increased risk of low birth weight, stillbirths, and/or neonatal mortality near cokeworks, and there was no strong evidence for any association between residence near cokeworks and postneonatal mortality. One must remember, however, the limited statistical power of the study to detect small risks.

Objective: In this study, we investigated whether low birth weight, stillbirth, or death up to an age of 1 y were more common among births to residents near cokeworks than those who lived in more-distant locations, independent of socioeconomic differences.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
? – based on distance from cokeworks	Longer term	Male and female	Perinatal to infant	n/a	n/a	n/a	n/a

Observations:

Observed/expected ratios for all sites were close to unity of the 0.7.5 km study area, except respiratory postneonatal mortality for which there was a statistically significant deficit. For all cokeworks combined, no excess was found within 2 km for low birth weight, still births, infant mortality, neonatal mortality, and respiratory postneonatal mortality. However, a general deficit of 25% was found in postneonatal respiratory mortality, compared with regional levels.

*Statistically significant

Author's Conclusions:

The authors concluded that there was no evidence of an increased risk of low birth weight, stillbirths, and/or neonatal mortality near cokeworks, and there was no strong evidence for any association between residence near cokeworks and postneonatal mortality. One must remember, however, the limited statistical power of the study to detect small risks

Review – Assessment

A. Subjects:	✎ Is the number of subjects sufficient to produce statistically significant results? + ✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study?+ ✎ Are the subjects a representative sample of the population to which extrapolations will be made?+ ✎ Were identical 'criteria for exclusion' applied to both cases and controls? NA ✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +
B. Exposure conditions:	✎ Was the exposure concentration measured?- ✎ Is the exposure duration known?-
C. Equipment:	✎ Was a full description of the monitoring devices provided?-- ✎ What was the sensitivity of the monitoring devices employed?-
D. Procedural:	✎ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? + ✎ Were appropriate controls recruited?+ ✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) - ✎ Was the timing and length of follow-up appropriate?+ ✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)+
E. Data collection:	✎ Was the assessment identical for cases and controls?+ ✎ Were appropriate endpoints examined?+ ✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom)+ ✎ Were accepted assessment methods employed?+ ✎ Were assessment measures sufficiently sensitive?+ ✎ Was the nature and severity of symptoms graded against an explicitly defined scale?+ ✎ Did qualified personnel conduct the exams?+ ✎ Was the timing and frequency of assessment logical?+ ✎ Was raw data provided to permit the reader to arrive at his/her own conclusions?- ✎ Was individual data reported for each subject?-

	✎ Are all withdrawals listed, with the reason for withdrawal?NA
F. Data analysis:	✎ Are the statistical methods used appropriate?+ ✎ Are confidence intervals reported?+ ✎ Did the investigator appropriately interpret the statistics?+ ✎ Are the observed trends statistically significant?+
G. Interpretations:	✎ Was the original objective addressed?+ ✎ Are the results novel?+ ✎ Was the design of the study appropriate to investigate the primary objective?+ ✎ Were assumptions reasonable?+ ✎ Are the methods reproducible?+ ✎ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)?+ ✎ Do the conclusions make biological sense?+ ✎ Are the extrapolations reasonable?+ ✎ Is the paper in a peer-reviewed journal?+ ✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document? +

Review - Summary:

Discussion of findings:

A statistical look at the effect of living close to cokeworks to infants, neonates and the fetus. In general the data was presented and processed very well but the objectives were to study the emissions of cokeworks and not specifically SO_2. No SO_2 (or other) contaminant concentrations were reported. Since no significant effect was found in general we can assume that the levels of SO_2 from cokeworks also pose no significant risks to fetuses, neonates and infants. However, it should be noted that some of the nonparametric statistical analysis were less powerful analysis tools, and may not have been able to detect small risks.

Confidence index:

The study used distance of residence from cokeworks as a proxy for SO_2 exposure; however, this may not be the best measure of exposure. No actual exposure measurements were made, and as such, the exposure calculations in this study may not be entirely valid. However, confounding variables were controlled for, and a detailed statistical analysis was conducted. However, statistical power of the study was not sufficient to detect small effects, as is noted by the authors. Some of the study's unexpected results may have been due to possibly erroneous assumptions of the study, such as the simple exposure model employed, that anticipated exposure to be higher near the site than at more-distant locations. Some impact of confounding variables such as certain socio-economic variables may also not have been controlled for.

Review – Confidence index ranking:

Low

Author: Franklin, C.A., Burnett, R.T.,Paolini, R.J.P. , Raizenne, M.E. | **Internal ID:** 004

Title:
Health Risks from Acid Rain: A Canadian Perspective

Year:1985

Abstract:

Acidic deposition, commonly referred to as acid rain, is causing serious environmental damage in eastern Canada. The revenues from forest products, tourism and sport fishing are estimated to account for about 8% of the gross national product. The impact on human health is not as clear-cut and a multi-department program on the Long-Range Transport of Airborne Pollutants (LRTAP) was approved by the federal government in June 1980. The objectives of the LRTAP program are to reduce wet sulphate deposition to less than 20 kg/ha per year in order to protect moderately sensitive areas. This will require a 50% reduction in Canadian SO_2 emissions east of the Saskatchewan/Manitoba border and concomitant reductions in the eastern U.S.A. The objectives of the health sector of the program are to assess the risk to health posed by airborne pollutants, which are subjected to long-range transport and to monitor the influence of abatement programs. Two major epidemiology studies were undertaken in 1983, one in which the health effects related to acute exposure to transported air pollutants were studied in asthmatic and nonasthmatic children, and another in which the effects of chronic exposure to these pollutants were studied in school children living in towns with high and low levels of pollutants. Preliminary analysis of the data do not indicate major health effects, but definitive conclusions must await final analysis. Studies on the indirect effects of acid deposition on water quality have shown that acidified lake water left standing in the plumbing system can adversely affect water quality and that federally set guidelines for copper and lead are exceeded. Flushing of the system before using the water rectifies the situation. Additional studies are planned to further delineate the magnitude of the health effects of acidified lake water.

Objective: Study 1: To study the health effects related to acute exposure to transported air pollutants in asthmatic and nonasthmatic children.
Study 2: To study the effects of chronic exposure to these pollutants in schoolchildren living in towns with high and low levels of pollutants.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Acute Study: Level for SO_2 not given **Chronic Study:** Average SO_2 concentration: Town 1:0 ppb Town 2:7.92ppb	**Acute Study:** 10 days **Chronic Study:** Not given	**Acute Study:** No info **Chronic Study:**No info	**Acute Study:** Children-no age info **Chronic Study**: 7-12 y	52 No info	**Acute Study:** 23 children were asthmatics **Chronic Study:** No Info	N/A	N/A

Observations:

Acute Study – Preliminary Results: There are no statistically significant observations reported regarding SO_2. There were no general observations reported for SO_2 specifically. Five asthma attacks occurred during the study interval. **Chronic Study – Preliminary Results** There are no statistically significant observations reported regarding SO_2. There were no general observations reported for SO_2 specifically.
*Statistically significant

Author's Conclusions:

"Acid deposition from both Canadian and American sources occurs in Canada. In the acute and chronic exposure studies conducted by the Department in 1983 and 1984, monitoring data showed that there was deposition of transported air pollutants and that there were both acute episodes of exposure at the summer camp as well as chronic exposure in Tillsonburg. Inconsistencies in the indoor-outdoor gradients indicate the need to obtain more precise information to enable accurate estimates of exposure. Preliminary analysis of the pulmonary function data from both the acute and chronic studies have not revealed any major detrimental health effects. However, certain trends in the data from the chronic study must be further analyzed before final conclusions can be drawn. It has been shown that acidification of lake water may lead to detrimental effects on water quality through leaching of metals from the plumbing system. The increased acidity may also result in mobilization of heavy metals and ultimate bioaccumulation of toxic metals such as mercury."

Review – Assessment:

A. Subjects:	(Acute Study: yes; Chronic Study: no numbers given) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (-) Were identical 'criteria for exclusion' applied to both cases and controls? (-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+/-) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval?

	(-) Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (-)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(-) Was the assessment identical for cases and controls? N/A Were appropriate endpoints examined? N/A Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) N/A Were accepted assessment methods employed? N/A Were assessment measures sufficiently sensitive? N/A Was the nature and severity of symptoms graded against an explicitly defined scale? N/A Did qualified personnel conduct the exams? N/A Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? N/A Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	N/A Are the statistical methods used appropriate? N/A Are confidence intervals reported? N/A Did the investigator appropriately interpret the statistics? N/A Are the observed trends statistically significant?
G. Interpretations:	N/A Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? N/A Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? N/A Do the conclusions make biological sense? N/A Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This paper uses two studies, one on acute effects of air pollution on lung function, the other on chronic effects to relate some of the studies being undertaken by LRTAP. Preliminary analysis of the pulmonary function data from both studies did not reveal major detrimental health effects. However, the purpose of the investigation does not appear to be to use individual study results for extrapolation purposes, but to emphasize that acidic deposition is occurring in Canada, and could have detrimental effects on may industries, as well as from a health perspective.

Confidence index:

The paper is slightly convoluted, in that it incorporates a literature review and two individual studies. Individual studies are briefly and incompletely addressed, with limited discussion of study design and limited mention of subject recruitment. The acute study only spanned 10 days. The chronic study was a cross-sectional design, which has a weak ability to prove causation. Sulfates are addressed by no information is given specifically for SO_2. Very limited raw data is provided from each study, preventing the reader from drawing independent conclusions on the results. There is no information on SO_2 levels, or any info linking SO_2 to health effects. This paper just provides preliminary results to studies that were meant to be ongoing. One literature review, and papers that focus on one of the studies done might better represent the information in this paper.

Review – Confidence index ranking:

Low

Author: Boezen HM, van der Zee SC, Postma DS, Vonk JM, Gerritsen J, Hoek G, Brunekreef B, Rijcken B, Schouten JP

Internal ID: 005

Title: Effects of ambient air pollution on upper and lower respiratory symptoms and peak expiratory flow in children

Year: 1999

Abstract: Background: Previous epidemiological studies have shown acute effects of increased amounts of ambient air pollution on the prevalence of respiratory symptoms in children with respiratory disorders. We investigated whether children with bronchial hyperresponsiveness (BHR) and relatively high serum concentrations of total IgE (>60 kU/L, the median value) are susceptible to air pollution.

Methods: We collected data from children during three winters (1992-95) in rural and urban areas of the Netherlands. Lower respiratory symptoms (wheeze, attacks of wheezing, shortness of breath), upper respiratory symptoms (sore throat, runny or blocked nose), and peak expiratory flow were recorded daily for 3 months. The acute effects of airborne particulate matter with a diameter of less than 10 um, black smoke, sulphur dioxide, and nitrogen dioxide were estimated by logistic regression.

Findings: 459 (73%) of 632 children had complete data. Of these, 26% had BHR and relatively high (above median) serum total IgE, 36% had no BHR and total IgE of 60 kU/L or less, 15% had BHR and total IgE of 60 kU/L or less, and 23% had a total IgE of more than 60 kU/L but no BHR. In children with BHR and relatively high serum total IgE the prevalence of lower respiratory symptoms increased significantly by between 32% and 139% for each 100 $\mu g/m^3$ increase in particulate matter, and between 16% acid 131% for each 40 $\mu g/m^3$ increase in black smoke, SO_2, or NO_2. Decrease in peak expiratory flow of more than 10% in that group was more common with increased airborne particulate matter and black smoke. There were no consistent positive or negative associations between increased air pollution and prevalence of respiratory symptoms or decrease in peak expiratory flow in the other three groups of children.

Interpretation: Children with BHR and relatively high concentrations of serum total IgE are susceptible to air pollution. Although our odds ratios were rather low (range 1.16-2.39) the overall effect of air pollution on public health is likely to be substantial since these odds ratios refer to large numbers of people.

Objective: To determine if children with BHR and relatively high serum level concentrations of total IgE (>60 kU/L, median value) are susceptible to air pollution.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
24 hr mean 8.3-22.5 $\mu g/m^3$ Median 7.4-21.6 $\mu g/m^3$ Median Range 0.6-61.3$\mu g/m^3$	Study took place over 3 winters for 3 months each	Male and Female	7-11 years	632 initially 459 with complete data	26% of 459 had BHR and high IgE 36% no BHR and low IgE 15% BHR and low IgE 23% no BHR and high IgE	N/A	N/A

Observations:

"Prevalence of lower respiratory symptoms increased significantly (by 16-31%) for every 40μg/m^3 increases in black smoke, SO_2, or NO_2 (lag through 5-day mean)."

SO_2 effects on risk of lower respiratory symptoms under 4 scenarios (Odds Ratios):
Lag 0: 1.44 (1.17-1.77)
Lag 1: 1.28 (1.00-1.64)
Lag 2: 1.38 (1.08-1.77)
5-day mean: 2.49 (1.54-4.04)

*Statistically significant

Author's Conclusions:

"We conclude that children with BHR and relatively high serum concentrations of total IgE should be targeted by public health-improvement studies. Medical characterization of subgroups of study populations seems worthwhile in future research into air pollution. Although odds ratios were rather low (range 1.16-2.39), the effect of air pollution is likely to be substantial since these odds ratios refer to large numbers of people."

Review – Assessment

A. Subjects:	+✓ Is the number of subjects sufficient to produce statistically significant results? +✓ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +✓ Are the subjects a representative sample of the population to which extrapolations will be made? +✓ Were identical 'criteria for exclusion' applied to both cases and controls? +✓ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	+✓ Was the exposure concentration measured? +✓ Is the exposure duration known?
C. Equipment:	-✓ Was a full description of the monitoring devices provided? -✓ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✓ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? +✓ Were appropriate controls recruited? -✓ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✓ Was the timing and length of follow-up appropriate? +✓ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✓ Was the assessment identical for cases and controls? +✓ Were appropriate endpoints examined?

	+✓ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✓ Were accepted assessment methods employed? +✓ Were assessment measures sufficiently sensitive? -✓ Was the nature and severity of symptoms graded against an explicitly defined scale? +✓ Did qualified personnel conduct the exams? +✓ Was the timing and frequency of assessment logical? -✓ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✓ Was individual data reported for each subject? -✓ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✓ Are the statistical methods used appropriate? +✓ Are confidence intervals reported? +✓ Did the investigator appropriately interpret the statistics? +✓ Are the observed trends statistically significant?
G. Interpretations:	+✓ Was the original objective addressed? +✓ Are the results novel? +✓ Was the design of the study appropriate to investigate the primary objective? +✓ Were assumptions reasonable? +✓ Are the methods reproducible? +✓ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings of this study seem reasonable, as the study utilized a large sample size and well-defined inclusion criteria. Several different components of air pollution were studied, albeit only in winter, and each had results quantified individually. Statistically significant results were only obtained for lower respiratory symptoms, and not for upper respiratory symptoms, or >10% PEF decrease in either mornings or evenings. Only those children that had BHR and relatively high serum total IgE did the prevalence of lower respiratory symptoms increase significantly for each 40um/m3 increase in SO_2.The results suggesting increased lower respiratory symptoms do appear to be of importance. The public health significance is that at-risk groups can be identified and targeted by public health-improvement strategies. The medical characterization of subgroups of study populations seems worthwhile in future research into air pollution.

Confidence index:

The study seems to be conducted rigorously in general, although trusting children to accurately report any symptoms they experienced into a diary over a three month period may not have been the best way of assessing health effects. The author did not mention the air monitoring instruments used in the study nor the sensitivity of the instruments. Subjects completed diaries independently, possibly introducing additional confounding factors into the study. In addition, the study did not appear to control for the lifestyles of the participants or medical history beyond BHR and serum concentrations. The children were chosen from rural and urban areas and SO_2 was measured continually at fixed sites in each urban and rural area. The exposure was measured at fixed sites but the approximate distance of the subject's homes, schools, etc. from the sites for the urban and rural areas were not measured. The sizes of the four groups were different (n=121, n=167, n=67, n=104).

It is difficult to determine whether any health effects were due to SO_2 exposure or other air pollutants, which were also measured in this study. Activity levels, amount of time spent indoors and outdoors, away from exposure etc. are not examined in this study. Justification is not provided for the (60kU/L) concentration chosen as "relatively high" IgE levels. Some of the methods and data are unclear. For example, for children with high BHR and IgE, it was stated that n=121 and there were 71 male and 59 female, however this adds up to 130.

Review – Confidence index ranking:

Moderate to high

Author: Ayres, J., Fleming, D., Williams, M. and McInnes, G.	**Internal ID:** 006

Title:

Measurement of Respiratory Morbidity in General Practice in the United Kingdom During the Acid Transport Event of January 1985

Year: 1989

Abstract:

The Weekly Returns System of the Royal College of General Practitioners was used to assess the effect of respiratory illness of the acid transport event that occurred during January 1985. The pollution event, as assessed by SO_2 and smoke levels measured at pollution monitoring stations within and without the affected area showed only modest rises in SO_2 levels, which were less than levels that occurred 4 years earlier. January is the peak time of year for reporting of acute respiratory episodes, and the minor increase in pollution was not reflected in any rise in respiratory morbidity, both for all ages and for different age bands. There was a rise in rates for children up to the age of 14, but this was seen each year and in both polluted and non-polluted areas. This was probably due to children returning to school after the winter vacation and the subsequent spread of viral infections. The limitations of the two data sets in this analysis are discussed, including the relative insensitivity of weekly data in picking out a short-lived event, the distribution of the practices and pollution monitoring stations, and the effect of the extreme cold weather and the coal miners' strike on domestic coal burning during this event.

Objective: To use two data sources (Weekly Returns System of the Royal College of General Practitioners Research Unit; Air Pollution Unit of the Warren Spring Laboratories) to assess whether or not the acid transport event of January 1985 produced a recognizable increase in acute respiratory illness in the affected area of the UK, and if weekly levels of SO_2 and smoke correlated with acute respiratory episodes during the three winter periods of December to February 1983-1984, 1984-1985 and 1985-1986.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Polluted area 49-104$\mu g/m^3$; Nonpolluted area: 33-72 $\mu g/m^3$	9 week study periods for 3 years	Male and female	All ages	Approx. 187000	-	-	-

Observations:

The mean daily level of SO_2 for the week of the pollution event was 104$\mu g/m^3$ in the polluted area and 72 $\mu g/m^3$ in the nonpolluted area. Equivalent levels for the two comparator periods were 66 and 49 $\mu g/m^3$ in 1983/1984 and 49 and 33 $\mu g/m^3$ in 1985 and 1986.

There were no significant differences in the size of populations at risk in the overall 9-week period in each of the three study periods.

Age-specific rates showed a marked rise in Total Respiratory Diseases (TRD) in the 5-14y age group and also, to a lesser degree, in the 0-4 age group. This appeared to coincide with the arrival of the pollution cloud in the year of the pollution episode but was seen in both polluted and nonpolluted areas for 1984-1985 and in the comparator years of study.

No rise was seen in acute bronchitis and acute asthmatic episodes during the polluted week in any age group. There were no differences either between polluted and nonpolluted areas or between the three study periods.

There was no correlation between SO_2 levels and TRD morbidity rates for all ages

*Statistically significant

Author's Conclusions:

"Using the data bases available, we have failed to show an increase in respiratory morbidity due to the acid transport event of January, 1985."

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+/-) Was the exposure concentration measured? (+/-) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (+/-) Were appropriate controls recruited? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	N/A Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+/-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed?

	(+) Were assessment measures sufficiently sensitive? (+/-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	N/A Are the statistical methods used appropriate? (-) Are confidence intervals reported? N/A Did the investigator appropriately interpret the statistics? N/A Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+/-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (+/-) Were assumptions reasonable? (+/-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? N/A Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Previous studies have linked respiratory morbidity with SO_2 exposure. The failure of this study to do so may be largely due to the design of the study and the many variables that were not controlled for. While the number of subjects involved in the study might suggest that results could be extrapolated, investigators did not sufficiently limit the confounding factors involved, potentially introducing significant error into the study. Furthermore, the authors failed to mention the statistical methods used and how and why they arrived at their endpoints (bronchitis and acute asthmatic episodes for example). Due to issues with the study design, lack of information etc, this paper does not provide information of any clinical significance.

Confidence index:

Several confounding factors were not considered during study design. For example, differences in climate over each of the three years may have affected the transport of SO_2 and affected the amount of domestic coal that was burned. Furthermore, no accurate information was given on what area the pollution cloud covered. In addition, weekly returns morbidity data would not have accurately depicted early peaks in data during the beginning of the week. In addition, investigators could not be sure which pollution monitoring sites subjects lived closest to, despite their preference for a particular clinic. Finally, investigators were not privy to the medical history of each of the subjects that may have contributed to pulmonary symptoms. The weekly returns system covered a wide variety of respiratory illnesses that were not necessarily related to SO_2 exposure.

Review – Confidence index ranking:

Low to moderate

Author: Donoghue AM, Thomas M	**Internal ID: 007**

Title: Point source sulphur dioxide peaks and hospital presentations for asthma

Year: 1999

Abstract:

Objective-To examine the effect on hospital presentations for asthma of brief exposures to sulphur dioxide (SO_2) (within the range 0-8700 μg/m^3 emanating from two point sources in a remote rural city of 25 000 people.

Methods-A time series analysis of SO_2 concentrations and hospital presentations for asthma was undertaken at Mount Isa where SO_2 is released into the atmosphere by a copper smelter and a lead smelter. The study examined 5-minute block mean SO_2 concentrations and daily hospital presentations for asthma, wheeze, or shortness of breath. Generalized linear models and generalized additive models based on a Poisson distribution were applied.

Results-There was no evidence of any positive relation between peak SO_2 concentrations and hospital presentations or admissions for asthma, wheeze, or shortness of breath.

Conclusion-Brief exposures to high concentrations of SO_2 emanating from point sources at Mount Isa do not cause sufficiently serious symptoms in asthmatic people to require presentation to hospital.

Objective: To examine the effect on hospital presentations for asthma of brief exposures to sulphur dioxide (SO_2) (within the range 0-8700 μg/m^3) emanating from two point sources in a remote rural city of 25 000 people.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0-8700μg/m^3	3 year period	Male & female	n/a	n/a	asthmatic	n/a	n/a

Observations:

No association was found between higher levels of SO_2 and incidence of hospital presentations or admissions.

*Statistically significant

Author's Conclusions:

"We conclude that brief exposures to high concentrations of SO_2 emanating from point sources at Mount Isa do not cause sufficiently serious symptoms in asthmatic people to require hospital presentation.

Review – Assessment:

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? ±✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study?

	+✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Were identical 'criteria for exclusion' applied to both cases and controls? +✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	+ Was the exposure concentration measured? +✎ Is the exposure duration known?
C. Equipment:	-✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+/-✎ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? -✎ Were appropriate controls recruited? -✎ Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) +✎ Was the timing and length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	±✎ Was the assessment identical for cases and controls? +✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? NA✎ Were assessment measures sufficiently sensitive? NA✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? -✎ Did either the study design or the statistical analysis limit confounding variables (*e.g.,* additional chemical exposure, etc.)?

	+✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The study did not find a positive association between point source SO_2 peaks and hospital presentations or admissions for asthmatic patients, in fact the available data suggested that significant negative correlations were present. No obvious reason for these results was present. Some points the authors make however, are that other potentially confounding airborne pollutants were not studied, and symptoms arising from elevated SO_2 levels may not have been sufficient for hospitalization, particularly given the availability of bronchodilators to remedy the symptoms at home. However, because of the many confounders not considered by the study, results should not necessarily be extrapolated to other situations.

Confidence index:

This population study appeared to be conducted in a rigorous manner, and advanced statistical methods were used to analyze data. However, the population-based correlation study design did not examine individual responses to changing concentrations of SO_2 in the air, which may have told a different story.
No distinction was made between subjects according to possible confounding factors such as age, smoking history or gender. This may have introduced a significant amount of error into study results. The study may have also been affected by the fact that respiratory response to SO_2 may be reversed with inhaled beta agonists, and therefore asthmatic people may not necessarily have come to the hospital during peaks in SO_2 exposure.

Review – Confidence index ranking:

Moderate

Author: Carnow, BW., Lepper, MH., Shekelle, RB. And Stamler, J. | **Internal ID:** 010

Title:
Chicago Air Pollution Study: SO_2 Levels and Acute Illness in Patients with Chronic Bronchopulmonary Disease

Year: 1969

Abstract:
A study was done to determine whether a dose-effect relationship exists between levels of pollution as measured by SO_2 and acute morbidity from respiratory disease in patients with chronic bronchopulmonary disease. For this purpose, a Registry of 561 patients with chronic bronchopulmonary disease was established. A graded pulmonary disease classification was developed, based on severity of symptoms; a daily personal pollution index was estimated for each patient, based on SO_2 levels in his square mile of employment and residence; and a daily calendar was maintained to record acute respiratory illness. In patients age 55 and over with grade 3 and 4 bronchitis, an increase in the acute morbidity rate was found in association with increased levels of SO_2. This increase in illness rate was recorded for the day of exposure and more consistently for the day following exposure.

Objective: The objective of the study was to determine whether a dose-effect relationship existed between levels of pollution as measured by SO_2 and acute morbidity from respiratory disease in patients with chronic bronchopulmonary disease.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Up to 0.30 ppm	Daily/10 months	Male and female	All	561	Diagnosed as having chronic bronchitis or chronic obstructive pulmonary emphysema	unknown	unknown

Observations:

For patients age 55 and over with chronic bronchitis grade 3 and 4, a definite association was demonstrable between level of SO_2 and percent person-days of illness. Rate of illness occurring on the same day as exposure to 0.25 ppm or more of SO_2 was 50% greater than rate associated with exposure to 0.04 or less.

For chronic bronchitis grade 3 and 4, age 54 or less, rate of illness did not rise steadily with increasing exposure to SO_2, but rates associated with the highest level of exposure, 0.30ppm or more, were considerably higher than rates associated with exposure to 0.04 ppm or less.

For chronic bronchitis grade 0 to 2, age 55 and over, no relationship was demonstrable. However, data for grade 0 to 2 bronchitis, age 54 or less again showed increasing rates of illness with increasing levels of SO_2.

For chronic bronchitis grade 3 and 4, age 55 and over, mean SO_2 levels during November and December were significantly higher for the day preceding illness than for the day preceding no illness. No statistically significant differences were demonstrable for other groups.
*Statistically significant

Author's Conclusions:

"Exacerbation of symptoms and precipitation of acute respiratory illness occur at high daily levels of SO_2 exposure among elderly persons with advanced bronchitis. It is also reasonable to conclude that the methodology of the Chicago Chronic Bronchopulmonary Disease Registry is generally sound and capable of adding meaningfully to knowledge on relationships between air pollutants and health."

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? N/A Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+/-) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (-) Were appropriate controls recruited? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	N/A Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+/-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? N/A Were assessment measures sufficiently sensitive? (+/-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical?

	(+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+/-) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (+) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This is an older study that examined the effects of SO_2 on a vulnerable population. The data was obtained with a combination of medical exams and questionnaires. The questionnaire was adequately designed with a scale for each reported symptomatic "event". Patient reporting was excellent at >90%. SO_2 was measured and a detailed description of the sampling times was noted. The results were statistically significant, showing a correlation between SO_2 and elderly persons with advanced bronchitis. Future investigations should examine health effects of other air pollutants in tandem with SO_2.

Confidence index:

In this study, description of monitoring devices was not provided, nor was the accuracy of the instruments reported. Future investigations of a similar nature might incorporate a control group to examine the effects of SO_2 on acute morbidity from respiratory disease in patients without chronic bronchopulmonary disease. Furthermore, there was no attempt to control for confounders such as smoking, activity level, occupational exposure etc. The symptoms were reported by the subjects each day on a calendar, but no other testing was done. The results of the study fully depend on the accuracy of the recording. Basically, the patients reported 'Chest Illness' or 'No Chest Illness'. (Initially, the patients maintained a record of dates of onset and duration of acute respiratory illness, emergency visits to physicians or emergency rooms, and hospitalizations. At the end of each month, these data were reported to the Chicago Chronic Bronchopulmonary Disease Registry. However, illness was reported more frequently near the beginning and end of the month than in the middle of the month.) Therefore, the analysis was limited to only three months when the calendar was used and 12 months of data was discarded. In addition, it is impossible to determine if other constituents of the air pollution were also affecting the health of the patients. Finally, the literature review included only 3 references.

Review – Confidence index ranking:

Moderate to low

Author: Cohen, AA., Nelson, CJ., Bromberg, SM., Pravda, M., Ferrand, EF. And Leone, G.

Internal ID: 011

Title:
Symptom Reporting During Recent Publicized and Unpublicized Air Pollution Episodes

Year: 1974

Abstract:
During the summer of 1970, New York City experienced a well-publicized period of moderately elevated air pollution, an unpublicized period of similarly elevated pollutant levels, and a period of low pollution. A telephone survey of irritative symptoms showed no significant differences between the publicized and the unpublicized episodes, but significant increases in eye irritation, throat irritation, chest discomfort, shortness of breath, restricted activity, and medical visits in adults during the two high pollution episodes. A low-pollution control area showed no significant symptom-rate variation with time.

Objective: While many researchers have attempted to define dose-response relationships of air pollutants and serious health effects, few have tried to define pollution levels at which significantly harmful symptoms first appear. The purposes of this study were to find whether moderate elevations of air pollution levels significantly increased the reported frequency of selected symptoms, to document any study bias introduced by widespread publicity about air pollution episodes, to describe who was most affected, and to sketch their pattern of symptoms.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.01-0.15 ppm	**Peak hourly**	**Both**	**Adult and Children (<21 y)**	**1121**	**NA**	**NA**	**NA**

Observations:

All symptoms except cough were significantly increased in the episode rates compared to that of the control period. When publicized and unpublicized episodes were compared, no significant differences were observed for any symptom. Reporting of symptoms was significantly more frequent in Queens (intermediate pollution) than in the Bronx (high pollution area) and both were higher than Riverhead (low pollution area). Symptom prevalence in the clean area (Riverhead) was lower than that in the polluted areas during the control period and the episodes. Chest burning was greatly increased in all exposed groups during the episodes. Consistent but less significant increases in cough, chest pain, shortness of breath, and restricted activity occurred in all exposed groups.

*Statistically significant

Author's Conclusions:

"The data indicate that irritative symptoms may significantly increase when sulphur dioxide levels exceed 0.11 ppm and suspended particulate levels exceed 145 µg per m^3 for several days. However, the possible confounding effects of other air pollutants, intercurrent infectious disease, and sociocultural differences could not be quantitated."

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (+) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	+Was the exposure concentration measured? +/- Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	+ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (+) Were appropriate controls recruited? (-) Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? + Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? + Are the observed trends statistically significant?

G. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? + Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (+) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results from this study indicated significant increases in physiological symptoms following air pollution episodes but no significant differences between publicized and unpublicized episodes. A control group showed no significant symptom-rate variation with time. However, many potentially confounding effects from factors such as unaccounted for air pollutants, intercurrent infectious disease and sociocultural differences raise questions about the potential for extrapolation of results from this study. However, the study did demonstrate, by indicating no significant difference between publicized and unpublicized episodes, that widespread publicity about an air pollution episode would not cause individuals to report more health symptoms. Nonetheless, higher overall pollution levels did increase reporting of symptoms.

Confidence index:

Confounders such as intercurrent infectious diseases and sociocultural differences may have affected this study. One apparent inconsistency of the study was higher symptom reporting from a community that exhibited lower SO_2 exposure levels. This may have had to do with sociocultural discrepancies between communities and different levels of environmental awareness. In addition, investigators did not retain control over who reported the symptoms. For example, for several symptoms, adult females reported higher levels than either adult males or young females. The author hypothesizes that this unexpected trend arose from the housewife responding on behalf of everyone in the home. A further potential complication with the study was placement of air monitoring equipment on buildings to prevent vandalism. This may have resulted in recordings of lower readings than what was at ground level. Moreover, it is impossible to study the effects of SO_2 independently or obtain individual exposure data given the study design.

Review – Confidence index ranking:

Moderate to low

Author: Buechley, RW., Riggan, WB., Hasselblad, V. and VanBruggen, JB.	**Internal ID:** 012
Title: SO_2 Levels and Perturbations in Mortality	
Year: 1973	
Abstract: Analysis of daily mortality for 422 places in the United States from 1962 to 1966 provided a consistent set of mortality predictors of three classes: annual cycle, day of week, and Christmas holidays; influenza epidemics; and days or spells of extreme cold or heat. Analysis of deaths for New York metropolitan region, with daily SO_2 measurements in Manhattan at the Davis Laboratory, showed the pollution measurement contributes significantly to prediction of deaths. Mortality was 1.5% less than expected on 232 days with SO_2 levels below 30 μg/m^3 and 2% greater than expected on 260 days with SO_2 levels above 500 μg/cu m, after correcting for the other factors. Coefficient of haze (COHS) does as well as SO_2 in predicting death. A parallel analysis in the Philadelphia area showed similar, but weaker, SO_2 and COHS effect.	
Objective: To assess the impact of air polluted with SO_2 on mortality rates in New York.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Ambient SO_2 levels; approximately 157 to 305 μg/m^3	1826 days	all	all	Populations from New York-New Jersey metropolitan area	-	-	-

Observations:

Results indicate a steady progression of mean mortality residuals as SO_2 values rise. Mortality was 1.5% less than expected on 232 days with SO_2 levels below 30 μg/m^3 and 2% greater than expected on 260 days with SO_2 levels above 500μg/m^3, after correcting for the other factors. The study also investigates the impact of other variables on mortality rate. Day of the weak is the weakest association with mortality, while influenza is the strongest.
*Statistically significant

Author's Conclusions:

"Analysis of deaths for New York metropolitan area, with daily SO_2 measurements in Manhattan at the Davis Laboratory, showed the pollution measurement contributes significantly to prediction of deaths. Mortality was 1.5% less than expected on 232 days with SO_2 levels below 30 μg/ m^3 and 2% greater than expected on 260 days with SO_2 levels above 500 μg/ m^3, after correcting for the other factors." In addition, "Coefficients of haze (COHS) does as well as SO_2 in predicting death. A parallel analysis in the Philadelphia area showed similar, but weaker, SO_2 and COHS effect."

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? N/A Were identical 'criteria for exclusion' applied to both cases and controls? N/A Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+/-) Was the exposure concentration measured? (-) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (-) Were appropriate controls recruited? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	N/A Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+/-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? N/A Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?

G. Interpretations:	(+/-) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+/-) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study found that SO_2 contributed to increased mortality rates in cities where other mortality predictors were in place. Based in New York, New Jersey and the Connecticut area, a study such as this could be extrapolated to other large urban areas where episodes of air pollution are problematic. However, since air pollution typically comprises more than SO_2, future studies might focus on the chemical make-up of smog and focus on its health or mortality rates.

Confidence index:

The study investigated a large number of subjects in the communities of New York-New Jersey-Connecticut. Because of the large sample size, it was difficult to limit confounding factors that may have impacted the study. This study used multiple regression in order to predict the contribution of SO_2 levels to mortality. The main limitation of this type of study as mentioned above is the possibility of confounders. This study was very simplistic and confounding variables would definitely be present. Examples are severe weather other than temperature, humidity, other pollutants (ozone, NO_2), just to name a few. Since the concentrations of SO_2 are typically low, the contributions due to acute exposure of SO_2 would also be small and the effect of confounding variables would inevitably mask such effects. Furthermore, there is uncertainty in the SO_2 measurements and therefore, the exposure of the population: Of all the monitoring stations in the metropolitan area only one monitoring station (Davis Laboratory) operated nearly continuously for the time period for which mortality is available. This data was still considered to be somewhat unreliable by the authors. The authors claim to have estimated SO_2 values for all days in 1962 to 1966 by various methods. There is absolutely no information for these methods. Therefore, any significant relationship between SO_2 and deaths is biased. I had answered 'Yes/No' to Are the statistical methods used appropriate, however, I believe the any statistics are meaningless due to the data collection methods. Literature review is not adequate (8 references) and no references for methods used to calculate SO_2.

The baseline value for the mean number of deaths per day was arbitrarily chosen to be the total number of deaths in five years (less disasters and time trends) divided by the number of days. The daily deaths were then compared to this number and the excess number of deaths determined. No justification was given for this calculation.

Review – Confidence index ranking:

Low to moderate

Author: Dockery DW. Ware JH. Ferris BG Jr. Speizer FE. Cook NR. Herman SM.

Internal ID: 013

Title: Change in pulmonary function in children associated with air pollution episodes.

Year: 1982

Abstract: Pulmonary function of approximately 200 school children in Steubenville, OH was measured before and immediately following air pollution alerts in the fall of 1978 and 1979. TSP concentrations exceeded the National Primary Ambient Air Quality 24h standards in 1978. SO_2 exceeded the standard in 1979. The children were then reexamined in 3 weekly visits following each alert. Estimated mean Forced Vital Capacity (FVC) was approximately 2% lower following each alert, although the lowest means were observed on to two weeks after the episodes. Forced Expired Volume in 0.75sec ($FEV_{0.75}$) did not change during the 1978 study, but was 4% lower immediately following the 1979 alert. The children were measured again in five weekly examinations in the spring and fall of 1980. Air pollution levels did not exceed the standards on either occasion. In the spring of 1980, estimated mean FVC and $FEV_{0.75}$ showed a decline similar to that observed following the alerts in 1978 and 1979. In the fall of 1980, there were no significant differences in the estimated mean FVC or $FEV_{0.75}$ between the examinations. A total of 335 children were tested in the four studies, including 194 who participated in more than one study. The evidence for each child from all the studies was combined in a regression analysis of pulmonary function on TSP and SO_2 average concentrations in the previous 24h. The distribution of the individual regression coefficients was centered significantly below zero, implying a decrease in pulmonary function with increasing TSP and SO_2 concentrations. The magnitude of the median change was less than 1% of the mean FVC and $FEV_{0.75}$ over the range of TSP and SO_2 concentrations observed.

Objective: **To examine changes in pulmonary function in children following air pollution episodes.**

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
169-455μg/m³	**Studied 24hr average at 4 points in time over a 2 year period**	**Male & female**	**3rd-5th grade (~8-10y)**	335	**n/a**	**n/a**	**n/a**

Observations:

Results are presented for each of several time periods, following both real and sham air pollution warnings:

1) Real warning: TSP 422μg/m³; SO_2 281μg/m³:
   - Immediately after: FVC -30mL from baseline ($p<0.01$)
   - week 1: FVC -44 mL from baseline ($p<0.001$)
   - week 2: FVC -52mL from baseline (significant)
   - week 3: FVC -33ml from baseline ($P<0.001$)

  - *Note $FEV_{0.75}$ was not significantly different from baseline at any time

2) Sham alert (TSP & SO_2 within guidelines) (interrupted by real alert)
    - Immediately after: FVC increased slightly, but non-significant
    - Week 1 interrupted by real alert (TSP 271μg/m^3 and SO_2 455 μg/m^3)
    - Week 1: FVC dropped significantly, $FEV_{0.75}$ dropped significantly ($p<0.01$)
    - Week 2: FVC increased slightly, but still significantly below baseline; $FEV_{0.75}$ still significantly below baseline
    - Week 3: FVC decreased again, $FEV_{0.75}$ still significantly below baseline
3) Sham Alert (TSP & SO_2 within guidelines)
    - Immediately after, and for weeks 1 & 2, FVC and $FEV_{0.75}$ significantly lower
4) Sham Alert (TSP & SO_2 within guidelines)
    - no significant differences in any weeks

A later regression analysis showed a relationship between FVC and SO_2 to be -0.057mL/μg/m^3 ($p<0.001$) (for $0 < SO_2 < 281$μg/m^3)

*Statistically significant

Author's Conclusions:

Repeated measurements of the lung function of 335 school children in Steubenville, OH has shown a statistically significant but physiologically small and apparently reversible linear decline in FVC and $FEV_{0.75}$ levels with increases in TSP and SO_2 24 h mean concentrations between approximately 0 and 275 μg/m^3. Although the data fit a linear model, a nonlinear or threshold model cannot be precluded within the range of exposures considered or at higher concentrations. The analysis reported has been limited to 24 h average concentrations. Investigations are continuing to determine if longer averaging times or maximum values over shorter averaging times have a higher association with change in lung function. Data are also available form the later studies for pollutants other than TSP and SO_2. These data are also being analyzed to rank pollutants in order of their association with lung function changes.

Review – Assessment

A. Subjects:	+✏ Is the number of subjects sufficient to produce statistically significant results? ±✏ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +✏ Are the subjects a representative sample of the population to which extrapolations will be made? ±✏ Were identical 'criteria for exclusion' applied to both cases and controls? +✏ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	+✏ Was the exposure concentration measured? +✏ Is the exposure duration known?
C. Equipment:	+✏ Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✏ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? +✏ Were appropriate controls recruited?

	-✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the timing and length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Was the assessment identical for cases and controls? +✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +*✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The study compared pulmonary function results following an episode of air pollution with pulmonary functions prior to an air pollution episode. This enabled investigators to compare episodic with baseline data, and permitted them to see changes that may have been caused by the air pollution episode. Results indicated small, but statistically significant decrease from baseline levels following the high pollution episodes in 1978 and 1979.

Confidence index:

While the study does produce statistically significant findings, there are some problems with the study must be noted. Some of the equipment used to monitor SO_2 concentrations in the study was noted to produce aberrant results, in some cases 15% lower than expected. While the problem was corrected shortly after, some data points previously collected may be incorrect. Also, because the study looked at TSP and SO_2 concurrently, it is difficult to isolate the independent effects of SO_2. The study occurred in a community with a read supply of resources including a pool of children experienced in performing the test, local people trained in pulmonary function testing and a reasonable probability of dramatic short term air pollution exposures. Evaluation of children's' lung function before an air pollution episode permitted use of this group as a control in further studies following episodes of air pollution. Overall the study design seems to be of good quality, with good controls, exposure measurements (for the most part), and good use of statistics to examine patterns in the data.

Review – Confidence index ranking:

Moderate to high

Author: Love GJ, Lan SP, Shy CM | **Internal ID: 015**

Title: A study of acute respiratory disease in families exposed to different levels of Air pollution in the Great Salt Lake basin, Utah, 1971-1972 and 1972-1973.

Year: 1982

Abstract: The reported incidence of acute respiratory illness in families exposed to different concentrations of air pollution was studied during two consecutive school years. The purpose of the study was to determine the effect of increased exposure to sulphur dioxide and suspended particulate matter. In each of four study communities, the mothers of approximately 250 white families were contacted biweekly to obtain information regarding the occurrence of respiratory symptoms in each family member. Annual mean ambient sulphur dioxide concentrations in one community for the three years included in the study (1971-1973) were well above the current air quality standard of 80 micrograms/m3, while in the other three communities the annual sulphur dioxide concentrations were much lower (usually less than 40 micrograms/m3). Suspended particulate matter concentrations in high sulphur dioxide community were close to the limit designated by the annual standard (75 micrograms/m3) but actual exposures in the four communities probably were not excessive. Regression analyses of the data collected showed inconsistent associations between illness rates and educational attainment of the head of household, crowding in the home, bronchitis in parents or smoking of parents. However, once the effects of these factors were removed the adjusted rates showed little association with community of residence. It was concluded that the higher concentrations of sulphur dioxide in the Utah atmosphere could not be the cause of increases in acute respiratory illness in the exposed populations.

Objective: The study investigated a single hypothesis that the incidence or severity of acute respiratory illness was associated with the concentration of air pollution to which populations were exposed.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
~10-120μg/m^3 annually	Daily – 3 years	Male & female	Elementary school children & their families	240-280 families/region	n/a	n/a	n/a

Observations:

Annual mean ambient sulphur dioxide concentrations in one community for the three years included in the study (1971-1973) were well above the current air quality standard of 80 μg/m^3, while in the other three communities the annual sulphur dioxide concentrations were much lower (usually less than 40 μg/m^3). Suspended particulate matter concentrations in the high sulphur dioxide community were close to the limit designated by the annual standard (75 μg/m^3) but actual exposures in the four communities probably were not excessive. Regression analysis of the data collected showed inconsistent associations between illness rates and educational attainment of the head of household, crowding in the home, bronchitis in parents or smoking by parents. However, once the effects of these factors were removed the adjusted rates showed little association with community of residence.

*Statistically significant

Author's Conclusions:

"The results of this study suggest that neither the incidence nor the intensity of reported respiratory illness was affected by exposure to mean annual concentrations of SO_2 in the range of 12-50% above the present annual air quality standard or $80\mu g/m^3$. The results leading to this conclusion, however, could have been affected by factors not controlled in this study, but this is not believed to be likely."

"In conclusion, it is believed that the data support the hypothesis that SO_2 in high concentrations apparently had little or no effect on the incidence of acute respiratory illness in Utah."

Review – Assessment

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +✎ Are the subjects a representative sample of the population to which extrapolations will be made? -✎ Were identical 'criteria for exclusion' applied to both cases and controls? +✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	+✎ Was the exposure concentration measured? +/-✎ Is the exposure duration known?
C. Equipment:	+✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✎ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? +✎ Were appropriate controls recruited? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the timing and length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Was the assessment identical for cases and controls? +✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +/-✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject?

	+/-✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? -✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? -✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results suggest that neither the incidence nor the intensity of reported respiratory illness was affected by exposure to mean annual concentrations of SO_2 in the range of 12 to 50% above the present annual air quality standard of 80 $\mu g/m^3$. This study is relevant because air quality standards continue to be controversial, and the option of increased use of native coal supplies should be continually reviewed.

Confidence index:

The study design is rigorous, with large sample sizes (>200) in each of several differently exposed regions. The study does well to make use of existing exposure conditions, and available data, while collecting new high quality data. The one potential weakness of the study is that the health effects studied were determined through self-reporting by telephone interview, without medical confirmation of symptoms; however this is unlikely to substantially change the study results. Air monitoring devices were atop buildings in two of the districts, and at ground level in two others, potentially introducing error into the study. In addition, the author suggests that results may have been affected by factors not controlled for including lack of independence among illness rates for various family members and the possibility that factors used in the regression analysis could be multicollinear. Also, there was no differentiation between illness due to SO_2 and illness due to other factors. An outbreak of influenza or colds would potentially skew the respondents in such a way to mask the effects of SO_2. No such reporting was done in the questionnaire to address these types of confounders.

Review – Confidence index ranking:

Moderate to high

Author: Holness, DL., Battend, B., Broder, I., Corey, P. and Mintz, S.	**Internal ID:** 016

Title: Evaluation of Respiratory Variables in Smelter and Control Workers Before and During a Shutdown Period

Year: 1985

Abstract: Thirty-six smelter workers examined in this pilot study were found to have a higher prevalence of cough and dyspnea and lower baseline lung function than did 31 controls. They also experienced decreases in forced vital capacity (FVC) and forced expiratory volume in 1s (FEV1) over the workweek while the controls did not. Baseline airflow rates and change in FVC and FEV1 over the workweek varied with levels of sulphur dioxide and particulates. Twenty-three smelter workers and 21 controls were seen on a second occasion, six months into an extended shutdown. The smelter workers continued to have a higher prevalence of cough and dyspnea and lower baseline lung function than the controls. There was, however, a slight increase in lung function in both the exposed workers and the controls during the shutdown. The results suggest that smelter workers may develop both acute and chronic work-related pulmonary effects and that the chronic effects may be nonreversible.

Objective: The objective of this pilot study was to determine whether nickel smelter workers differ from controls in their respiratory systems and pulmonary function, whether these differences are stable or reversible over a six-month period of absence from work, and whether there are exposure-effect relationships between SO_2 or particulate exposure and decreased pulmonary function.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Average 0.47 ppm (smelter workers)	2 weeks	male	Approx 26-53	67	53% of smelter workers are smokers; 52% of controls are nonsmokers	unknown	unknown

Observations:

The higher prevalence of dyspnea in the smelter workers was statistically significant even after adjustment was made for number of pack-years of smoking by logistic regression analysis (P=.04).

The smelter workers had consistently lower values of baseline FVC, FEV_1, and flow rates, which remained after correction for smoking by multiple regression analysis.

The smelter workers showed a decrease in their FVC and FEV_1 from Monday morning to Thursday afternoon while the controls did not. A reverse trend occurred for the flow rates. The percentage decrease in FVC from Monday to Thursday was significantly larger for the smelter workers than for the controls.

The smoking-adjusted prevalence of respiratory symptoms did not change appreciably among either the smelter workers or the controls between their June and December examinations. The prevalence of cough and shortness of breath was significantly higher among the smelter workers. The Monday morning pulmonary function results, again adjusted for smoking, tended to rise similarly in both groups during the period of shutdown but remained relatively lower in the smelter workers.

*Statistically significant

Author's Conclusions:

"The results suggest that smelter workers may develop both acute and chronic work-related pulmonary effects and that the chronic effects may be nonreversible."

"The flow rates of our population of smelter workers tended to change from Monday to Thursday in an opposite direction to the FVC and FEV_1. This apparent paradox could be explained on the basis of the flow measurements being made at a higher lung volume when the FVC decreases, assuming that there has been an associated increase in the residual volume with the total lung capacity remaining constant."

"There is evidence from this pilot study that smelter workers may develop both acute and chronic work related effects on their pulmonary function. The chronic effects may be nonreversible."

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (+) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(+) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (+) Were appropriate controls recruited? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams?

	(+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+/-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results from this study suggest that smelter workers may develop both acute and chronic work-related pulmonary effects and that the chronic effects may be nonreversible. While these results are opposite to a those from a study that examine lung function in 17 normal subjects, the sustained difference between the two is consistent with a nonreversible component related to employment as a smelter worker. The results are conclusive for exposure in smelter conditions; however, the experimental design does not fully prove that SO_2 was the cause of the observed effects. For example, nickel and other heavy metals would be expected to be at very high concentrations but levels were not reported. This is a major omission by the authors.

Confidence index:

This study incorporated a control group and made use of an appropriate sample size. Control and test subjects underwent identical testing. Furthermore, recruitment method was outlined and described reasons for subjects withdrawing from the study. Possible confounding factors such as age, height and weight were accounted for. Furthermore, smoking was adjusted for in the analysis. The environmental monitoring system was described in detail and confidence intervals were given.

Review – Confidence index ranking:

Moderate

Author: Likas C. Exarchou V. Gourgoulianis K. Giaglaras P. Gemptos T. Kittas K. Molyvdas PA.	**Internal ID: 017**
Title: Noxious gases in greenhouses.	
Year: 2001	
Abstract: The concentration of NO(2) and SO(2) was measured in a commercial greenhouse from 23/9/1999 25/01/2000. The measurements showed that the level of the two gases is very high in the greenhouse atmosphere. Lung function tests in 42 workers showed that temporary work did not influence significantly the respiratory health status.	
Objective: "The aim of this study was the estimation of SO_2 and NO_2 levels in a greenhouse environment"	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.15-0.66ppm	Mean hourly/ avg. duration 6.6years	22 Males & 20 females	mean = 34.2	42	n/a	0.66ppm mean hourly	n/a

Observations:

The mean hourly concentration for SO_2 ranged from 0.15-0.66 ppm. Highest average values were recorded at 15:00. The number of subjects displaying FEV_1 <80% of the predicted values was 3, while no subjects demonstrated FEV_1 <60% predicted values.
*Statistically significant

Author's Conclusions:

"Healthy workers usually show no changes in lung function following temporary exposure. Nevertheless, the values of NO_2 and SO_2 measured in our study could cause respiratory symptoms, especially in asthmatics."

Review – Assessment

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Were identical 'criteria for exclusion' applied to both cases and controls? ±✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?

B. Exposure conditions:	+✎ Was the exposure concentration measured? +✎ Is the exposure duration known?
C. Equipment:	+✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? -✎ Were appropriate controls recruited? -✎ Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) +✎ Was the timing and length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	±✎ Was the assessment identical for cases and controls? +✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? -✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	-✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? -✎ Did the investigator appropriately interpret the statistics? -✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? -✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? -✎ Did either the study design or the statistical analysis limit confounding variables (*e.g.,* additional chemical exposure, etc.)? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

These findings primarily provide estimates of exposure to SO_2 and NO_2 in greenhouses. While 42 greenhouse workers participated in a questionnaire and medical exam, the evaluation of respiratory health effects was secondary in this study. No effects were found.

Confidence index:

This paper was presented as a brief communication. No mention made of how subjects were recruited. Furthermore, the author does not mention if there were any withdrawals, and if so, what there reasons were for not participating. No control groups were incorporated into the study design to allow for comparison of SO_2 and NO_2 effects separately. Finally, it is unclear whether subjects were exposed to gases for 8 hours straight, or whether they were given breaks throughout the course of the day. Sample sizes were relatively small, and no statistical analyses of collected data were carried out. The findings of this study are generally of low confidence. On the whole, the measurement of SO_2 in greenhouses is useful, however the clinical data in this paper is highly suspect.

Review – Confidence index ranking:

Low to Moderate

Author: Hoek, G., Brunekreef, B.	**Internal ID:** 018

Title:
Acute Effects of a Winter Air Pollution Episode on Pulmonary Function and Respiratory Symptoms of Children

Year: 1993

Abstract: The acute respiratory effects of a wintertime air pollution episode were studied in a general population sample of 112 children who were 7-12 years of age and who lived in a nonurban community. Spirometry was performed on 6 d, with a fixed interval of 3 wk between successive tests. During air pollution episode, an additional pulmonary function test was made. Acute respiratory symptoms of the children were noted in a diary. Ambient concentrations of sulphur dioxide, black smoke, particulate matter with an aerodynamic diameter less than 10 μm, and nitrogen dioxide were considered as exposure variables. The association of air pollution with pulmonary function and prevalence of acute respiratory symptoms was assessed by individual linear regression analysis and time series analysis, respectively. In February 1991, an air pollution episode occurred during which daily average sulphur dioxide concentrations were slightly above 100 μg/m^3, and particulate matter (with an aerodynamic diameter of less than 10 μm) concentrations reached 174 μg/m^3. During the episode, forced vital capacity, forced expiratory volume in 1 s, and maximal midexpiratory flow were lower than on baseline tests. Significant negative associations were found between the concentration of sulphur dioxide, black smoke, and particulate matter with an aerodynamic diameter of less than 10 μm. No association between prevalence of acute respiratory symptoms and the concentration of these compounds was found.

Objective: To investigate possible relationships between air pollution and respiratory symptoms. Previous studies have observed decreased pulmonary function of subjects during wintertime air pollution episodes, where daily SO_2 concentrations were in the range of 200-250 μg/m^3.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0-100 μg/m^3	Daily x 14 days	male/female	7-12	112	healthy	unknown	unknown

Observations:

During an episode of elevated air pollutants, subjects' pulmonary function appeared to be lower than that measured on several baseline test days. However, after adjusting for ambient temperature, there was no association between prevalence of acute respiratory symptoms and air pollution concentrations.

Highest concentration levels of particulate matter >10μm, black smoke and SO_2 were observed during a 14-day interval in February, 1991. SO_2 concentrations were below the suggested WHO guideline. At 6d, PM_{10} concentrations were above the LOAEL suggested by WHO (110 μg/m^3).

Association between air pollution and acute respiratory symptoms apparent from simple tabulation was no longer present after adjustment for ambient temperature.

*Statistically significant

Author's Conclusions:

"Significant negative associations were found between the concentration of sulphur dioxide, black smoke, and particulate matter with an aerodynamic diameter of less than 10 μm."

"Direct effects of SO_2 on pulmonary function of healthy children are highly unlikely at the concentration levels observed during this study.

It is not clear which components of the episode mix were responsible for the observed associations."

"No association between prevalence of acute respiratory symptoms and the concentration of these compounds [sulphur dioxide, black smoke, PM_{10}] was found."

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(+) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+/-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (-) Were appropriate controls recruited? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+/-) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+/-) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+/-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive?

	(+/-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+/-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+/-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (+/-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study finds no association between prevalence of acute respiratory symptoms and concentration of air pollutants once ambient temperature is corrected for. While ranges of exposure for each pollutant is referred to, this study does not report LOAEL or NOAEL. While results are not novel, they contradict other studies that indicate decreased pulmonary function during wintertime air pollution episodes. Discrepancies between results from this study and other studies might be due to inadequate characterization of air by examining only a few indicator pollutants.

Confidence index:

Because the study examined respiratory response to ambient air pollutant levels, no follow-up was required. In addition, while the study attempted to correct for confounding factors (including level of activity, respiratory health etc.) no control groups were used since the study was conducted *in vivo*, and controlling the emission of air pollutants would have been impossible. It is difficult to characterize the actual exposure of SO_2 by fixed site monitoring of ambient SO_2 concentrations. Furthermore, the pulmonary function tests were performed every 3 weeks (6 days), but more frequent testing would have provided a more reliable baseline. It is difficult to assess the effects of SO_2 from an air pollution mixture.

Review – Confidence index ranking:

Moderate

Author: Harkonen H. Nordman H. Korhonen O. Winblad I. | **Internal ID: 021**

Title: Long-term effects of exposure to sulphur dioxide. Lung function four years after a pyrite dust explosion.

Year: 1983

Abstract: The lung function of 7 men accidentally exposed to sulphur dioxide (SO_2) in a pyrite dust explosion was followed for 4 yr. The greatest decrease in forced vital capacity, forced expiratory volume in one second, and maximal midexpiratory flow was observed 1 wk after the accident. After about 3 months no further decrement occurred. The pattern of spirometric findings was obstructive in 6 and restrictive in 1 of the patients. Four years after the accident a reversible obstruction of the bronchi was still observable in 3. Four patients reacted positively to the histamine challenge test. Two patients either did not respond to bronchodilator or did not react to histamine. The results suggest that bronchial hyperreactivity is a frequent sequela after exposure to high concentrations of SO_2. The hyperreactivity may persist for several years.

Objective: n/a

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
? – exposed to gases in a mine explosion	**Single exposure: 20-45min**	**male**	**22-34**	**7**	**? – presumed healthy**	**n/a**	**n/a**

Observations:

Immediately following exposure, thoracic pain, coughing, conjunctival irritation, and in come cases, corneal erosion were noted

The greatest decreases in FVC, $FEV_{1.0}$, and MMEF were witnessed 1 week after the accident.

Lung function parameters increased during the 4 years of follow-up, but never back to pre-accident predicted values.

Also, 4 of 7 still showed bronchial hyperreactivity in the histamine provocation test 4 years after the accident.

*Statistically significant

Author's Conclusions:

"…it seems justified to regard SO_2 as the principal cause of pulmonary injury in the subjects described in this report."

Review – Assessment

A. Subjects:	-✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? -✎ Are the subjects a representative sample of the population to which extrapolations will be made?

	-✎ Were identical 'criteria for exclusion' applied to both cases and controls? +✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	-✎ Was the exposure concentration measured? +✎ Is the exposure duration known?
C. Equipment:	-✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✎ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? -✎ Were appropriate controls recruited? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the timing and length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+/-✎ Was the assessment identical for cases and controls? +✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✎ Was individual data reported for each subject? +✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +/-✎ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? +✎ Do the conclusions make biological sense?

	+✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings are based on a small case report of 7 individuals who were exposed to SO_2 and other gases in a coal-mining incident. While follow up for the study was 4 years, and the methods involved seem to be of good quality, no statistical measures were provided for analyzing the findings, nor were exposure levels provided. While this study relied on a mine accident for its results, measurements of pulmonary response to acute exposure to SO_2 may be extrapolated to similar circumstances in the future, to suggest effective rehabilitation methods for subjects, and to anticipate pathology arising from the exposure.

Confidence index:

This study was a case report with only 7 cases (small sample size), limited exposure concentration information, with confounding factors that could not be limited and no statistical analysis. From a clinical standpoint, no new data was gathered as symptoms of acute SO_2 exposure were well known and reproduced in this study. For an epidemiological standpoint, the test sample size was too small to adequately assess long-term effects. At most, the report provides anecdotal evidence for some of the clinical aspects of acute SO_2 exposure.

Review – Confidence index ranking:

Low

Author: Kesten S. Szalai J. Dzyngel B	**Internal ID: 023**
Title: Air quality and the frequency of emergency room visits for asthma	
Year: 1995	

Abstract: BACKGROUND: Atmospheric pollution has been proposed as one of the possible factors responsible for increases in asthma mortality and morbidity. **OBJECTIVE:** We sought to examine whether we could demonstrate a relationship between emergency room visits for asthma and alterations in environmental conditions. **METHODS:** Over a 1-year period, the frequency of emergency room visits for asthma in a large urban hospital were documented and compared to outdoor concentrations of SO_2, NO_2, and ozone in addition to two overall measures of air quality (air pollution index and air quality index). **RESULTS:** A total of 854 emergency room visits were noted with the highest number of visits occurring in May and between September and December. Significant variations in the frequency of visits as well as environmental conditions could be seen on a daily basis. Despite comparisons of results on a daily, weekly, and monthly basis, no significant relationships could be found between any of the pollution indices and emergency room visits. Staggering visits by 1 and 7 days, however, revealed a relationship between emergency room visits and air pollution index and air quality index. An association between emergency room visits and NO_2 and ozone was seen when visits were staggered by 7 but not by 1 day. **CONCLUSIONS:** We conclude the fluctuations in overall air quality are associated with increased frequency of emergency room visits but only when data are lagged by a predefined period.

Objective: We sought to examine whether we could demonstrate a relationship between emergency room visits for asthma and alterations in environmental conditions.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
~0.00-1.5 ppm	1-year study	Male & female	?	854 emergency room visits	Asthmatic	n/a	n/a

Observations:

"When the indices air pollution index and air quality index were lagged by one day behind the number of visits and 20 autocorrelation terms were incorporated in the model, both air pollution index and air quality index indices yielded significant regression coefficients ($p=0.007$, and $p=0.044$) respectively. When the past 20 visits were used in the predictive autoregression in addition to air pollution index and air quality index, the R^2 increased from 2.3% to 11.5%"

*Statistically significant

Author's Conclusions:

We conclude the fluctuations in overall air quality are associated with increased frequency of emergency room visits but only when data are lagged by a predefined period.

Review – Assessment

A. Subjects:	+✐ Is the number of subjects sufficient to produce statistically significant results? +/-✐ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +✐ Are the subjects a representative sample of the population to which extrapolations will be made? +/-✐ Were identical 'criteria for exclusion' applied to both cases and controls? +✐ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	-✐ Was the exposure concentration measured? +/-✐ Is the exposure duration known?
C. Equipment:	-✐ Was a full description of the monitoring devices provided? -✐ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✐ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? -✐ Were appropriate controls recruited? -✐ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✐ Was the timing and length of follow-up appropriate? +✐ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+/-✐ Was the assessment identical for cases and controls? +✐ Were appropriate endpoints examined? +/-✐ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✐ Were accepted assessment methods employed? +✐ Were assessment measures sufficiently sensitive? +/-✐ Was the nature and severity of symptoms graded against an explicitly defined scale? +✐ Did qualified personnel conduct the exams? +✐ Was the timing and frequency of assessment logical? - Was raw data provided to permit the reader to arrive at his/her own conclusions? -✐ Was individual data reported for each subject? +/-✐ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✐ Are the statistical methods used appropriate? -✐ Are confidence intervals reported? +✐ Did the investigator appropriately interpret the statistics? +/-✐ Are the observed trends statistically significant?
G. Interpretations:	+✐ Was the original objective addressed? -✐ Are the results novel?

	+✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? -✎ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The authors do not find a correlation between SO_2 levels in air pollution and emergency room visits. They do, however, note an association between several indices of air pollution and air quality with emergency room visits. While air pollution does apparently increase the frequency of emergency room visits, the same cannot be said for SO2 alone from this study. While this information would be valuable to predict increases in emergency room visits during air pollution episodes, the many confounding factors in the study prevent reliable extrapolation to other similar scenarios.

Confidence index:

This population-level study uses hospital data. Environment Canada Pollution reports to investigate the link between emergency room visits for asthma and air pollution. This study however recorded very little about the subjects involved. For example, age range was not indicated, nor was socioeconomic background or medical history. Exposure and outcome data cannot be linked at the individual level with this study design, and could represent a reason for the lack of significant results. Furthermore, the concentrations of SO_2 studied in this paper may be too low to result in emergency room visits for asthma. This paper's main focus is not on SO_2 but on air quality in general. It makes no attempt to separate the effects of individual chemicals in an air mixture. No statistical data or interpretation on the link of SO_2 and emergency room visits was given except to say that it was monitored. There is almost no value in this paper for the dissemination of information about SO_2. Therefore the conclusions able to be drawn from this study may be somewhat limited.

Review – Confidence index ranking:

Moderate to Low

Author: Vigotti MA. Rossi G. Bisanti L. Zanobetti A. Schwartz J. **Internal ID: 027**

Title: Short term effects of urban air pollution on respiratory health in Milan, Italy, 1980-89.

Year: 1996

Abstract: STUDY OBJECTIVE: To investigate the association between daily urban air pollution and acute effects on respiratory health. **STUDY DESIGN:** Time series analysis following the procedure defined in the APHEA protocol. **SETTING:** City of Milan, Italy, from 1980-89. Two air pollutants, total suspended particulates (TSP) and sulphur dioxide (SO_2), and two health outcomes, deaths and hospital admissions were considered. The last was analysed according to two age groups. **SUBJECTS:** Daily deaths and general hospital admissions for respiratory causes in residents who died in Milan or were admitted to local hospitals in that city. **MAIN RESULTS:** There was an increased risk of respiratory death and of hospital admission associated with increased concentrations of SO_2 and TSP. The relative risks were similar for both pollutants, and were higher for respiratory deaths than for hospital admissions. No changes in relation to season were seen in the SO_2 effect on respiratory deaths, but there was a suggestion of a higher effect on hospital admissions in the cool months. The seasonal pattern of the TSP effect was inconsistent: for mortality it was higher in the warm period while for hospital admissions it seemed to be higher in the cool months. This last result might be due to chance, although some role could have been played by the hospital admission data on all general admissions for respiratory causes (ICD-9: 460-519) as these are a much less specific end point. **CONCLUSION:** In Milan, a positive association was found between the daily SO_2 or TSP concentrations and the number of deaths or hospital admissions for respiratory causes. This confirms results from other European and North American cities.

Objective: To investigate the association between daily urban air pollution and acute effects on respiratory health.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
3.0 -827.8 µg/m^3	**24 hr mean / 10 years**	**Male & female**	**All ages**	**Study population ~1.5 million**	**Studied respiratory admissions/deaths**	**n/a**	**n/a**

Observations:

Deaths

"We found a statistically significant positive effect of SO_2 exposure on the number of deaths that occurred on the same day (RR=1.12, 95%CI 1.03,1.23)… The average exposure to SO_2 on the same and the previous day had a slightly larger effect size (RR=1.16, 95%CI 1.05, 1.29)… No relevant difference has been observed in the SO_2 effect when the level of particular matter air concentration is above or below 100µg/m^3."

Hospital Admissions

"In those aged between 15 and 64 years the best fitting model for the 24 hour SO_2 exposure was the same day, while for the cumulative exposure it was the average of four consecutive days. The effect of SO_2 exposure, of the single day or the cumulative days, was more pronounced in the cool months, although the interaction term testing seasonal differences was not significant. No evidence of interaction with different levels of TSP was found."

"In the older group (65+), the best fit in the models for SO_2 exposure was found at the same lags as the younger group. The associations were slightly weaker, however."

*Statistically significant

Author's Conclusions:

"We found that both SO_2 and TSP concentrations were associated with increased risks of respiratory deaths and hospital admissions… We found an increase of 4-5% in hospital admissions for all respiratory causes for a $100\mu g/m^3$ increase in the TSP or SO_2 concentration… For SO_2 the mortality association showed no effect in relation to season, but there was evidence of such effect for hospital admission; the association seemed stronger in the winter."

Review – Assessment

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Were identical 'criteria for exclusion' applied to both cases and controls? +✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	+✎ Was the exposure concentration measured? -✎ Is the exposure duration known?
C. Equipment:	+✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? -✎ Were appropriate controls recruited? -✎ Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) +✎ Was the timing and length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Was the assessment identical for cases and controls? +✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? -✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate?

	+✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
G. Interpretations:	+✏ Was the original objective addressed? +/-✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective? +✏ Were assumptions reasonable? +✏ Are the methods reproducible? +✏ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The authors find that SO_2 independently has an effect on the number of respiratory hospital admissions and deaths. The findings are based on rigorous statistical analyses, and actual dose-response relationships are observed. These findings appear to be useful and of high quality. Consequently, results may be extrapolated to similar urban centers to estimate effects of air pollution on residents.

Confidence index:

The catchment area for the hospitals used in this study is very large (>1.5million people), and the study was carried out for ten years. Thus the large sample size, and long-term data collected are likely to provide very high quality results and minimize the effects of stochastic variation on study results. A rigorous statistical analysis was used to examine potentially confounding factors including air temperature and season, and good exposure data were collected.

The study suffers from not being able to control confounding factors such as smoking or socioeconomic factors. In addition, hospital admission procedures, dependent on cultural differences and national medical practices, may have influenced baseline number of admissions and estimated relative increase. Finally, it is difficult to record how long each subject was exposed to pollution in a day, making dose-effect relationships difficult to judge.

Review – Confidence index ranking:

Moderate

Author: Lawther PJ. Brooks AG. Lord PW. Waller RE — **Internal ID: 029**

Title: Day-to-day changes in ventilatory function in relation to the environment. Part I. Spirometric Values.

Year: 1974

Abstract: Measurements of forced expiratory volume at 1 second, forced vital capacity, and maximum midexpiratory flow have been made on four normal subjects employed in central London on each working day for five years. For three of the subjects, MMF was highest on days with low concentrations of smoke and sulphur dioxide, and it decreased at successively higher concentrations, but it was not clear whether this was an effect of pollution or of other associated factors. Respiratory infections were found to have pronounced effects on these spirometric measurements, and analysis of the results for the complete series revealed small seasonal variations and long-term changes, some of which were large and in the opposite direction from those expected with increasing age. Some immediate but transient effects of pollution on the spirometric values were seen following exercise in periods of high pollution. In addition to small direct effects, the role of pollution in affecting the course of respiratory infections and thus indirectly further affecting ventilatory function was considered.

Objective: n/a

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Up to 5600μg/m^3	**Daily measurements for 5 years (1960-65)**	**Male**	**30-41**	**4**	**Healthy**	**n/a**	**n/a**

Observations:

Among the four study subjects the authors noted several patterns, though all of the subjects did not necessarily share them:

1) "On visual comparison, it seemed that decreases in FEV1 and MMF were associated with the increases of pollution and other seasonal factors."

2) "The measurement showing the most consistent association with pollution was MMF..."

3) IN ONE SUBJECT: "The best evidence of pollution effects was obtained during episodes of high pollution, when supplementary measurements of ventilatory capacity were made during the day… Although concentrations of pollutants were high indoors as well as outdoors, there was no change in ventilatory capacity during normal activities in the laboratory. Outdoor exercise led to some reduction: FEV1 fell by 5%after a short walk or run in the very high concentrations of smoke and sulphur dioxide found in the streets at that time, and MMF fell by 15%. There was no appreciable change in FVC. Similar changes were seen in other periods of high pollution and in other subjects, but in all cases the effects could be detected only if measurements were made within minutes of returning from outdoor exercise."

*Statistically significant

Author's Conclusions:

"Whilst there was seen to be an inverse relationship between FEV_1 and pollution in one subject, and between MMF and pollution in three, we are not sure that this was a direct effect. The finding that exercise was necessary to elicit any effect of high pollution in these normal subjects, and that even then the effect was transient, led us to think that the normal morning measurements were unlikely to reflect variations in pollution. The greatest effects were observed when concentrations of smoke and sulphur dioxide were high, and when the subject exercised sufficiently to demand deep breathing by mouth."

Review – Assessment

A. Subjects:	-✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? -✎ Are the subjects a representative sample of the population to which extrapolations will be made? -✎ Were identical 'criteria for exclusion' applied to both cases and controls? ±✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	+✎ Was the exposure concentration measured? -✎ Is the exposure duration known?
C. Equipment:	-✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? -✎ Were appropriate controls recruited? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the timing and length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+/-✎ Was the assessment identical for cases and controls? +✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? ±✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? ±✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel?

	+✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? -✎ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings are based on 4 study subjects followed daily for 5 years. While some apparent patterns are noted, they vary greatly by individual. No tests of significance are mentioned, nor are confidence intervals provided. It is likely that trends cannot be detected in such a small sample size. Extrapolation of these results, despite the fact that 800 sets of measurements were taken should not be extrapolated since the 4 subjects in this sample were not representative of the larger population. Furthermore, the authors were unable to distinguish to which degree certain airborne pollutants may have been responsible for the observed effects. No strong conclusions about SO_2 effects on human health can be drawn from this paper.

Confidence index:

The study uses only 4 individuals, though over a long period of time, to obtain large numbers of measurements. However, individual variation appears to outweigh any shared effects of pollution. This variability combined with a lack of statistical analyses result in this study having low confidence. No mention is made of how subjects were recruited, or reasons for withdrawal, if any at all. Furthermore, no attempts were made to discern the effects of individual pollutants.

Review – Confidence index ranking:

Low to moderate

Author: Lawther, PJ., Brooks, AGF., Lord, PW. And Waller, RE.	**Internal ID:** 030

Title: Day-to-Day Changes in Ventilatory Function in Relation to the Environment Part II: Peak Expiratory Flow Values

Year: 1974

Abstract: Measurements of peak expiratory flow have been made on four normal subjects on each working day for 5 years. Although there was no clear relationship between day-to-day changes in peak flow and environmental factors, analysis of the results over the whole period showed small seasonal variations for each subject, with lower values on the colder, more polluted days in winter. A gradual increase rather than the decrease expected with age was found during the 5 years of the study. Multiple regression analysis on the combined results for the four subjects showed that after removing the trend with time the environmental factor explaining the largest proportion of the residual variance was the concentration of sulphur dioxide. The decrease in peak flow corresponding with a large increase in sulphur dioxide concentration, however, was small, and much of the day-to-day variation in peak flow was due to factors other than the concentrations of smoke or sulphur dioxide, temperature, or relative humidity. Reductions in peak flow were often associated with respiratory infections, and, as in the parallel study of spirometric measurements, the possibility that pollution had indirect effects on ventilatory function by affecting the course of respiratory infections was considered.

Objective: To record the peak flow measurements from four subjects in relation to the environment.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Ambient concentrations	5 years	male	30-41	4	Healthy	unknown	unknown

Observations:

Each subject showed a highly significant ($p<0.001$) increase in peak flow during the 5 year series (1960-1965) and a fall in the 5 year interval before further measurements were made.

Seasonal variations in peak flow did not follow a consistent pattern, but when results for all 5 years were combined, winter means were slightly but significantly lower than summer ones.

In all the subjects, peak flow increased as pollution diminished, and in three of them, peak flow increased as temperature decreased.

For all subjects, the mean peak flow decreased in successive ranges of sulphur dioxide concentrations, although anomalously on a few isolated days of very high pollution.

Daily variations in peak flow were small and not clearly related to changes in pollution.

*Statistically significant

Author's Conclusions:

"Present analyses have shown that small direct effects of environmental factors on peak flow are detectable in a long series of measurements, and pollution appears to be more important than temperatures or relative humidity. The results of the measurements of peak flow reported in the present paper show that direct effects of pollution on this relatively crude measure of ventilatory function were small. Day-to-day changes were affected more by respiratory infections, although it was possible that pollution affected the frequency or course of these. These points had already been noted when a preliminary analysis of results was done halfway through the study, and for the final two years of it, from 1963-65, one of the subjects made five additional sets of peak flow readings each day in a further attempt to separate effects of exposure to pollution from variations due to other factors. Also, from 1965, peak flow measurements were made twice daily for a period of 5 years on one bronchitic subject.

From 1965, however, the daily measurements of spirometric and peak flow values on normal subjects were discontinued in favour of measurements of airways resistance. The results of a pilot study on one subject, referred to above, had shown that airways resistance, as determined in a body plethysmograph, provided a more sensitive measure of day-to-day changes in lung function in relation to environmental factors than did the simple spirometric and peak flow measurements. These studies continued on three normal subjects for more than 5 years, and further papers on the results of airways resistance measurements, and on the remaining series of peak flow measurements, are in preparation.

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+/-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (-) Were identical 'criteria for exclusion' applied to both cases and controls? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (-) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (-) Were appropriate controls recruited? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	N/A Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and

	dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? N/A Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+/-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+/-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results from this study indicate that direct effects of pollution on this relatively crude measure of ventilatory function were small. Because the study involved only four subjects, it is difficult to state whether this represents an actual trend, or is just a result of a very small sample size. Results of this nature do not show strong statistical significance.

Confidence index:

While investigators indicated the device used to measure peak expiratory flow rate, it was not described, nor was mention made of its accuracy rate. The study made use of only four subjects, over a five-year study period, without continuously tracking medical history and affects of aging. During this length of time, other confounders that may have impacted the study were the implementation of the Clean Air Act. How the subjects were recruited was similarly not mentioned, not was any withdrawals.

Peak flow collected in weekly means. No daily information was given. Furthermore, it is stated that many series of experiments on the effects of inhaled pollutants and occasionally these exposures appeared to have some effect on the daily measurements of peak flow, however, details of these experiments are not given and are presumably included in another study.

Out of the 1250 working days in the series, each subject had been available on about 800 days but on only 351 of these were measurements made on all four. The peak flow changes were different for each of the subjects.

Review – Confidence index ranking:

Low to moderate

Author: Lawther PJ. Brooks AG. Lord PW. Waller RE.	**Internal ID: 031**
Title: Day-to-day changes in ventilatory function in relation to the environment. Part III. Frequent measurements of peak flow	
Year: 1974	
Abstract: As an extension of an earlier study of day-to-day variations in peak expiratory flow, measurements were made on two normal subjects before and after their daily journey to work, and at three times during the day. For one of these subjects there was some correlation between the change in peak flow on entering London each morning and the concentration of sulphur dioxide at the time, but in both the main feature was a consistent diurnal variation. As in earlier studies, respiratory infection was found to be the main factor causing reductions in the daily mean peak flow, while stress led to some increases in values. Peak flow measurements were also made twice daily on two bronchitic subjects and for one of them there was a relationship between the values at the end of the working day and the concentrations of sulphur dioxide (serving as an index of pollution in general). This relationship disappeared, however, when the clinical condition of the subject changed, and no consistent relationship with pollution could be found with results form the other subject.	
Objective: n/a	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Up to 1600μg/m^3	**Daily – up to 2 years**	**Male**	**35-62**	**4**	**2 Healthy** **2 bronchitic**	**n/a**	**n/a**

Observations:

Healthy subjects "There was no significant correlation between the peak flow on arrival at work each day (PEF_2) and the corresponding pollution index, but for REW (one of the study subjects) there was a significant, though small, correlation between the magnitude of the increase (of respiratory function during the day) whilst travelling to work (ΔPEF) and pollution. This increase was smaller on days of high pollution than on days of low pollution, and for the 2 year period as a whole the regression equation was: ΔPEF liters/minute = $24.8 - 7.07\log_{10} SO_2$ μg/m^3. However, this relationship accounts for only 2.6% of the variance in ΔPEF and the equation is a poor predictor of the increase on any given day. Using this equation, the difference in ΔPEF between a day with low pollution (SO_2 1000μg/m^3) would be 9.2 liters/minute (1.5% of the mean PEF), but the standard error of this estimate is 12.5 liters/minute." Bronchitic subjects "…the highest correlation coefficient between PEF_2 and the pollution index was for subject HT during the first 2 months: the correlation was negative, there being relatively low peak flow values on days of high pollution ($p<0.01$). The correlation between log SO_2 and the change in peak flow during the working shift ($\Delta PEF = PEF_2 - PEF_1$) was lower than that with PEF_2. For the other subject (RR) none of the correlation coefficients was significant."
*Statistically significant

Author's Conclusions:

"In this final paper of the trio reviewing the results of several long series of daily measurements of forced expiratory volumes and peak expiratory flows made in London since 1960, a little more evidence has been presented to show that pollution may have some effects, but it is clear now that many other factors affect day-to-day and even hour-to-hour changes in these measures of ventilatory capacity, and often these can be of overwhelming importance. One of the most interesting findings reported in the present paper was the close relationship between peak flow and pollution in one of the bronchitic subjects on first entering the series, with the disappearance of this response after a period of illness that appeared to be precipitated by high pollution. Although it is only one example, this result follows the same pattern as findings from studies of subjectively assessed changes in the condition of large groups of bronchitic patients. Among these patients, periods of high pollution lead to immediate exacerbations of their bronchitis: many remained ill for days or weeks afterwards and in the course of each winter studied, the response of the patients to pollution appeared to die away as more of them became ill or remained under treatment with antibiotics and bronchodilator drugs. The best way of combining the two types of study may then be to select moderately impaired subjects, and to make daily measurements of peak flow among them for a limited period only, at the beginning of a winter.

Review – Assessment

A. Subjects:	-✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? -✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Were identical 'criteria for exclusion' applied to both cases and controls? +✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	+/-✎ Was the exposure concentration measured? -✎ Is the exposure duration known?
C. Equipment:	-✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? -✎ Were appropriate controls recruited? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the timing and length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	±✎ Was the assessment identical for cases and controls? +✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and

	dose-response relationship provided for each symptom) +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? +-✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✏ Was individual data reported for each subject? ±✏ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✏ Are the statistical methods used appropriate? -✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? ±✏ Are the observed trends statistically significant?
G. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective? +✏ Were assumptions reasonable? +✏ Are the methods reproducible? -✏ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results indicated that for normal subjects, respiratory infection was found to be the main factor causing reductions in daily mean peak flow, while stress led to some increases in values. In bronchitic subjects, a relationship was seen between the values at the end of the working day and the concentrations of sulfur dioxide. However, this relationship disappeared when clinical conditions of the subject changed, and no consistent relationship with pollution could be found with results from the other subject. As expected, with such a small sample size, no significant results could be found. However, the results of the findings supported and extended the findings reported in Part II of the study. It should be noted, however, that while sulfur dioxide was used as the index of pollution in this part of the study, that it was not necessarily responsible for any of the effects observed. It is equally likely that pollutants such as sulphuric acid may have been important.

Confidence index:

This study based on 4 subjects, two healthy males, and two bronchitic males followed for up to two years for their ventilatory responses to SO_2 and other pollution. However, several problems exist with this study that question its confidence:

1) the healthy subjects were considerably younger than the bronchitic ones (35 & 44 as opposed to 59 & 62, respectively)
2) exposure assessment was based at a single location, and could not actually measure individual exposures
3) each subject displayed different responses, making generalizations difficult

Nevertheless, due to the rigorous nature of the data collection, this study would justify future similar studies with many more subjects and a set of control subjects.

Review – Confidence index ranking:

Low

Author: Amdur, MO., Melvin, WW., Drinker, P.	**Internal ID:** 032
Title: Effects of Inhalation of Sulphur Dioxide by Man	
Year: 1953	
Abstract: Dense concentrations of sulphur dioxide produced during the "London Fog" of December, 1952 precipitated a study of the respiratory response to concentrations of sulphur dioxide. Subjects were exposed to varying concentrations of SO_2 released into a chamber and their expired air was recorded by a spirometer. Respiratory symptoms began at approximately 5ppm and included changes to tidal volume, respiratory rate and pulse-rate.	
Objective: To examine possible respiratory responses of subjects to low concentrations of SO_2.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1-8 ppm	10-minute intervals	male	28-58	14	Healthy	-	1 ppm

Observations:

No statistically significant observations made

Fourteen people exposed to sulphur dioxide at 1-8 ppm showed changes in respiration and pulse-rate. Tidal volume decreased, respiratory rate increased, minute volume remained unchanged and pulse rate increased.

Two additional men who worked daily in atmospheres containing sulphur dioxide 10 or more ppm showed no changes when exposed to 5 ppm.

*Statistically significant

Author's Conclusions:

"Shallower and more rapid respiration and increased pulse-rate were observed during exposure [1-8 ppm SO_2]. Men who worked daily in atmospheres containing SO_2 10 or more ppm showed no changes when exposed to 5 ppm."

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an

	unrepresentative sample population? (-) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+/-) Was evidence provided to justify the dosing regime? (-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (-) Was a control group assayed? N/A Is the control regime appropriate? (-) Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+/-) Was the length of follow-up appropriate? (+/-) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+/-) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+/-) Were accepted assessment methods employed? (+/-) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-) Are the statistical methods used appropriate? (-) Are confidence intervals reported?

	(-) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+/-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+/-) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (+/-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Investigators determined that SO_2 levels as low as 1 ppm can produce shallow rapid respirations and increased pulse-rate. Furthermore, it was established that respiratory and cardiac responses to SO_2 were less in people regularly exposed to it than in those people not previously exposed to it. While trends are evident in the data, in terms of respiration rates and volumes, as well as pulse rate, the sample size is small and no statistical analyses were conducted. The findings appear to make biological sense, but are not scientifically rigorous.

Confidence index:

Sample size for this study (14) was too small to produce statistically significant results. Statistical methods were not reported. The process of recruitment was not given for this study, nor was mention made of people who chose to be excluded from the study. The study included male subjects only, preventing extrapolation of results to females. In addition, smoking history was not given. No controls were incorporated into the study design. Finally, no follow-up was mentioned for the subjects who participated in the study. The olfactory detection of SO_2, which was variable among participants, may have biased the results. The figures only showed the average values of tidal volume, respiration rate and pulse rate for five of the fourteen participants (Fig1) and four of the fourteen participants (Fig2). It is reported that the same patterns of change were followed by each person, however, it is not clear if the authors mean each of the five people or each of the fourteen participants. Also, no explanation is given for why all fourteen individual's data was not included. Another figure compares only two of the participants each exposed to a different level of SO_2 (2ppm and 1ppm).

To determine whether the same effects would be obtained from people regularly breathing atmospheres containing SO_2, only two men from an industrial plant were exposed to 5ppm. These results are not meaningful or useful due to the small number of participants and high variability among participants.

Review – Confidence index ranking:

Low to moderate

Author: Speizer FE, Frank NR	**Internal ID: 033**
Title: The uptake and release of SO_2 by the human nose.	
Year: 1966	
Abstract: (SUMMARY) Measurements were made of the absorption and desorption of sulphur dioxide in the upper respiratory tracts of seven healthy men breathing by nose. Virtually all of the inspired sulphur dioxide was absorbed by the nasal mucosa. It was not possible to saturate the system during the period of study at the concentrations used. During expiration sulphur dioxide was desorbed from the nasal mucosa in quantities which totaled approximately 15% of the original concentration inspired. An implication of this study is that chemically minute amounts of sulphur dioxide (less than 1% of the inspired concentration) are, upon reaching the larynx and more distal airways, sufficient to initiate reflex changes in bronchomotor tone.	
Objective: To determine the extent of uptake of SO_2 by the human nose in healthy volunteers	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
16.1ppm	**Up to 5 times: 25-30 min**	**male**	**27-39**	**7**	**healthy**	**n/a**	**n/a**

Observations:

SO_2 concentration decreased upon inspiration by subjects: - Initial concentration in the breathing mask was 16.1ppm - 1-2cm inside the nose concentration fell to 13.8ppm - oropharynx – SO_2 too small to be measured (0.3ppm – control was 0.2ppm) - expired gas in the pharynx virtually free of SO_2 (0.4ppm vs. 0.3ppm in the control) - expired gas exiting the nose picked up SO_2 (2.0ppm) "Symptoms.- Most patients felt slight irritation of the posterior pharynx during only the first few minutes of exposure."
*Statistically significant

Author's Conclusions:

"Virtually all of the SO_2 in inspired air was removed by the nose during exposures lasting 30 minutes. There was no systematic difference between absorptive rates for the first and second halves of exposure."

"During exposure to the gas, about 12% of the amount taken up by the tissues in inspiration reentered the air stream in expiration."

Review – Assessment

A. Subjects:	-✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate

	for the objective of the study? +/-✎ Are the subjects a representative sample of the population to which extrapolations will be made? +✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +/-✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? +✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? -✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale?

	+✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? -✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This paper focused on the mechanism of uptake of SO_2 by the respiratory tract. The instrumentation appears to be crude, however the authors seemed careful to control important factors when performing the SO_2 measurements. The results appear to be significant as the results are very striking in nature. The results presented are useful for looking into the mechanism of absorption of SO_2. This data can be used to design future study into clinical treatment strategies for people exposed to SO_2 or help in explaining epidemiological data.

Confidence index:

Although the sample sizes are a bit small (7), the study produced statistically significant results. The methods of the study seem to be of good quality, and the data collected seem to be appropriate. Although statistical tests were not used, confidence intervals were provided to justify conclusions. While subjects were classified as healthy, and their ages were listed, no other information was given for each subject, including medical background. This is a reproducible study, and descriptions of monitoring devices were specific and included a diagram.

Review – Confidence index ranking:

Moderate to high

Author: Winterton DL. Kaufman J. Keener CV. Quigley S. Farin FM. Williams PV. Koenig JQ

Internal ID: 035

Title: Genetic polymorphisms as biomarkers of sensitivity to inhaled sulphur dioxide in subjects with asthma.

Year: 2001

Abstract: BACKGROUND: Individuals with asthma are sensitive to inhaled sulphur dioxide (SO_2); decrements in pulmonary function occur after exposure to low concentrations even for a short duration of time. There is a great amount of interindividual variation in response to SO_2. **OBJECTIVE:** It was our objective to determine whether one of the following polymorphism locations linked with asthma is associated with the bronchial hyperresponsiveness to SO_2 observed in some asthmatic patients: the beta2-adrenergic receptor, interleukin-4 (IL-4) receptor alpha subunit, Clara cell secretory protein (CC16), TNF-alpha gene promoter, and first intron of the lymphotoxin alpha (LT-alpha) gene. **METHODS:** Subjects were volunteers with physician-diagnosed asthma requiring regular asthma medication. Spirometry was performed before and after a 10-minute exposure to 0.5 ppm SO_2. Subjects were classified as SO_2 responders if forced expiratory volume in 1 second (FEV1) decreased > or = 12%. DNA obtained from buccal cell samples was analyzed for genetic polymorphisms. **RESULTS:** Of the 62 subjects (21 male and 41 female), 13 had a 12% or greater decrement in FEV1 after SO_2 exposure (range + 19% to -49%). Response to SO_2 was associated with the wild-type allele of the TNF-alpha promoter polymorphism (12 of 12 SO_2 responders versus 28 of 46 nonresponders; $P < .05$) but with no other polymorphisms. Medication category and atopic status showed no association with SO_2 sensitivity. **CONCLUSIONS:** The wild-type allele of the TNF-alpha promoter polymorphism may be associated with mechanisms of asthmatic sensitivity to inhaled SO_2.

Objective: It was our objective to determine whether one of the following polymorphism locations linked with asthma is associated with the bronchial hyperresponsiveness to SO_2 observed in some asthmatic patients: the beta2-adrenergic receptor, interleukin-4 (IL-4) receptor alpha subunit, Clara cell secretory protein (CC16), TNF-alpha gene promoter, and first intron of the lymphotoxin alpha (LT-alpha) gene.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.5ppm	**10min**	**Male & female**	**18-55**	**62**	**Asthmatic**	**n/a**	**n/a**

Observations:

"A significant association was observed between SO_2 response and the homozygous wild-type allele of the TNF-α position -308 (OR = infinity; adjusted OR=16.25, CI 1.5-infinity). This allele was observed in all 12 of the responders to SO_2, whereas the mutant and heterozygous alleles were present in 18 of the non-responders."

*Statistically significant

Author's Conclusions:

"This study found a significant association between response to SO_2 and the homozygous wild-type allele of TNF-α(TNF-1). None of the other polymorphisms showed significant trends."

"A primary limitation of this study was the small sample size (n=61). This limitation was even more evident in the analyses with stratifications by atopy status and medication use. With a larger sample size, the association between TNF-α and sensitivity to SO_2 seen in this study could be affirmed."

Review – Assessment:

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +✎ Are the subjects a representative sample of the population to which extrapolation will be made? +✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? ±✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? -✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale?

	+✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? +✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? ±✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

These findings show a link between SO_2 responsiveness and the TNF-α gene. The findings could be useful for predicting responsiveness to SO_2 among individuals in the future. Findings indicate that the wild-type allele of the TNF-alpha promoter polymorphism may be associated with mechanisms of asthmatic sensitivity to inhaled SO_2. Because of the relatively small sample sized used (61), association between SO_2 and TNF-alpha cannot be affirmed. Furthermore, with a larger sample size, some of the polymorphisms tested in the study may have shown an association with SO_2 sensitivity.

Confidence index:

The methods of exposure, subject recruitment, and data collection all seem to be very good. The sample is relatively large, and gender-balanced as to be more representative of the population from which it was drawn. Sample size could have been larger to provide for better stratification and analysis of individual genes, but all in all the study seems to be of very good quality. The experimental design was adequate in studying the objective. The molecular biology appeared to successfully find all the candidate polymorphisms. There was one important limitation in this study that may make the data questionable. The use of FEV as an end-point is valid yet it was the ONLY criteria used to determine if a subject was a responder or nonresponder. This is troubling from a few points of view. First, the authors mentioned that the subjects were all on a variety of different medications, which could influence the response to SO_2. Second, individual differences (age, height, weight etc.) are likely to influence FEV results. Third, other end-points could have been easily incorporated into the experimental design as corroborating data. Although the results are interesting, a follow-up study is warranted which could look directly at the identified potential genetic polymorphisms with examination of multiple end-points.

Review – Confidence index ranking:

Moderate to high

Author: Koenig, JQ., Pierson, WE., Horike, M. and Frank, R.	**Internal ID:** 038

Title: Bronchoconstrictor responses to Sulphur Dioxide or Sulphur Dioxide Plus Sodium Chloride Droplets in Allergic, Nonasthmatic Adolescents.

Year: 1982

Abstract: Eight atopic adolescent subjects without diagnosis of clinical asthma but with signs of hyperactive airways were studied. The subjects were exposed for 30 minutes at rest followed by 10 minutes during moderate exercise on a treadmill to the following: (1) filtered air, (2) 1 mg/m^3 NaCl droplet aerosol, (3) 1 ppm SO_2 and NaCl droplet aerosol, or (4) 1 ppm SO_2. All exposures were at 75% relative humidity and 22°C. Exposures to either SO_2 mode produced statistically significant changes in pulmonary function, whereas sham exposures to air on NaCl did not. These results are similar to those seen earlier in a group of extrinsic asthmatic adolescent subjects and are three to 2 times greater than changes we saw in a group of normal adolescent subjects. thes changes seen after inhalation of SO_2 were not statistically different from those seen after inhalation of SO_2 and NaCl droplet aerosol. Our results indicate that inhalation of 1 pm SO_2 by a group of atopic adolescents can produce exercise-induced bronchospasm at a level of exercise that has no effect by itself.

Objective: To examine bronchoconstrictor responses to sulphur dioxide or sulphur dioxide plus sodium chloride droplets in allergic, nonasthmatic adolescents.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1 ppm	30 min at rest, 10 min while exercising	male and female	12 to 18	8	Clinical diagnoses of allergic rhinitis concomitant with sinusitis or atopic dermatitis but without extrinsic asthma.	unknown	1 ppm

Observations:

There were no statistically significant differences from baseline values in pulmonary function after exposure for 30 minutes at rest to any of the four exposure modes.

Filtered Air Alone/NaCl alone: Exposure to either filtered air alone or filtered air plus NaCl alone during moderate exercise resulted in no significant changes from baseline values in pulmonary functional measurements.

SO_2 and NaCl. Four of the five pulmonary functional measurements showed statistically significant changes compared with baseline means after exposure to SO_2 and NaCl droplet aerosol during moderate exercise. R_T (total respiratory resistance) increased, FEV_1 (forced expiratory volume in 1 s) decreased and V_{max50} (max flow calculated at 50% vital capacity) and V_{max75} (Maximum flow calculated at 75% vital capacity). Functional residual capacity did not change significantly.

SO_2 alone: Three functional measurements showed statistically significant changes compared with baseline means after exposure to SO_2 alone during moderate exercise. FEV_1 decreased, V_{max50} and V_{max75} were reduced. R_T and FRC did not change significantly.

SO_2 and NaCl compared with SO_2 alone: The magnitude of change from baseline values seen after SO_2 and NaCl droplet aerosol exposure after exercise was compared with the degree of change from baseline after SO_2 exposure for each of the functional parameters. There were no statistically significant differences between the responses to the two SO_2 exposure modes based on this comparison.

*Statistically significant

Author's Conclusions:

The extrinsic asthmatic subjects were slightly more responsive to the SO_2 and NaCl droplet than were the atopic subjects; however, they both were substantially more responsive than were healthy adolescents.

The statistical comparisons between SO_2 exposures and SO_2 and NaCl exposures showed no significant differences in any of the pulmonary functional measurements.

The present study demonstrated that atopic adolescents without clinical diagnoses of asthma are much more sensitive to the effects of inhaled SO_2 mixtures than are healthy adolescents.

It appears that in the atopic adolescents, exposure to SO_2 alone or SO_2 and NaCl droplet aerosol produced similar effects.

Our findings of marked sensitivity to SO_2 in both asthmatic adolescents and nonasthmatic atopic adolescents indicate that outdoor activity in the presence of SO_2 may produce an adverse effect in these individuals.

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+/-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided?

conditions:	(-) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+) Was a full description of the monitoring devices provided? N/A What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+/-) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (-) Was a control group assayed? N/A Is the control regime appropriate? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? N/A Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate?

	(-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results indicated that inhalation of 1 pm SO_2 by a group of atopic adolescents can produce exercise-induced bronchospasm at a level of exercise that has no effect by itself. However, due to the low number of subjects involved in the study, results cannot be interpreted as statistically significant. Also, this study should have used a control group: non-atopic, non-asthmatic adolescents. The authors compared the results from this study to another which had data to this end, however, it is difficult to evaluate the other data without full disclosure of the experimental design and results. Nonetheless, results were unique in that they determined that SO_2 alone, rather than as part of a mixture (with NaCl) produced bronchial symptoms. These findings may be important for a very specific subpopulation but can not be generalized to other populations. Various pulmonary functions were significantly affected by SO_2 exposure in these adolescents. This study at least warrants further investigation.

Confidence index:

As mentioned, this study employed too few experimental subjects. This study did not mention how subjects were recruited, nor did it limit confounding factors such as smoking or previous medical history (excluding asthma). Difficult to generalize this data to other populations. The subjects were classified as being clinically diagnosed with allergic rhinitis concomitant with sinusitis or atopic dermatitis but without extrinsic asthma, a very specific population. Conditions among the subjects varied slightly. For example, six of the subjects showed positive responses to a methacholine challenge test; one showed a negative response and one was not tested. The authors later conclude that the subjects may be classified as having subclinical asthma.

The exposures were only partially randomly chosen. Since all subjects had EIB (exercise induced bronchoconstriction), the first exercise session on the treadmill was always during exposure to filtered air. Exposure to SO_2 was always the last exposure.

Review – Confidence index ranking:

Moderate to low

Author: Kreisman H. Mitchell CA. Hosein HR. Bouhuys A.	**Internal ID: 039**
Title: Effect of low concentrations of sulphur dioxide on respiratory function in man.	
Year: 1976	
Abstract: Respiratory function was evaluated in human subjects breathing SO_2 (0.5-5.0ppm) by mouth for 1-5min. A variable response of maximal respiratory flow rates was obtained at rest. Light exercise potentiated the effect of 3.0ppm whereas premedication with propranolol or indomethacin did not. Symptoms were consistently observed on exposure to 3.0ppm or greater. These data suggest that re-evaluation of the present threshold limit value is indicated.	
Objective: "We studied the effect on human respiratory function of breathing SO_2 in concentrations of 0.5ppm-5.0ppm for short periods. We also studied its possible modification by light exercise and by the prior administration of a prostaglandin-synthetase inhibitor, indomethacin, or a β-adrenergic blocker, propanolol.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.5-5.0ppm	**1-5min**	**n/a**	**22-34**	**18**	**Healthy**	**0.5ppm**	**1.0ppm**

Observations:

"We have observed significant changes in MEF 40%(P) after inhalation of small concentrations of SO_2 by mouth for 1-5minutes. However, several months later, repeat inhalation of 1.0ppm at rest did not produce a significant change. The response to SO_2 (3.0ppm) was enhanced by exercise but not by either propanolol or indomethacin."

"The airway response to SO_2 inhalation varied greatly in this study. Several subjects whose airways initially responded to 1.0ppm were not affected by this or high concentrations later on. The inability to reproduce our initial data and the absence of a dose-response relationship after increasing concentrations of SO_2 confirm the variable airway response to inhalation of this gas."

"Light exercise potentiates the airway effect of SO_2... More than 70% of our subjects had symptoms after a 3-minute exposure to SO_2 concentrations as low as 3.0ppm during rest or light exercise. Fifteen reported dryness, irritation or burning sensation in the throat, and 12 of them also had pulmonary signs and symptoms…"

"The negative results with propanolol and indomethacin suggest that neither β-adrenergic stimuli nor prostaglandin release play a demonstrable role in the action of SO_2. Atropine inhibits the constrictor response to SO_2, and effect which can be explained either by the reflex hypothesis or by interaction between physiological cholinergic stimuli and SO_2 at the level of the effector tissue."

*Statistically significant

Author's Conclusions:

"We feel that the current threshold limit value for SO_2 (5.0ppm) should at least be lowered to 3.0ppm, a concentration which leads to symptoms and function changes in many healthy persons even after brief exposures, as in the present study."

Review – Assessment:

A. Subjects:	-✎ Is the number of subjects sufficient to produce statistically significant results? +/-✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study?

	+/-✎ Are the subjects a representative sample of the population to which extrapolations will be made? +/-✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +/-✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? +/-✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +/-✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? +/-✎ Are all withdrawals listed, with the reason for withdrawal?

F. Data analysis:	+✓ Are the statistical methods used appropriate? +✓ Are confidence intervals reported? +✓ Did the investigator appropriately interpret the statistics? +✓ Are the observed trends statistically significant?
G. Interpretations:	+✓ Was the original objective addressed? +✓ Are the results novel? +✓ Was the design of the study appropriate to investigate the primary objective? +✓ Were assumptions reasonable? +✓ Are the methods reproducible? +✓ Did either the study design or the statistical analysis limit confounding variables? +/-✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review – Summary:

Discussion of findings:

This study found responses in subjects to exposures of SO_2 upwards of and including concentrations of 1 ppm. This study was interesting as it repeated the testing several months later using a concentration of 1 ppm. A great deal of individual variation was noted, leading to what may appear to be inconsistencies in the results. The findings do suggest that persons can respond to low concentrations of SO_2 (1.0ppm) and that the effects are potentiated by exercise, though individual responses vary considerably. Exposures to 3.0 ppm SO_2 produce pulmonary symptoms. These findings are similar to those from other studies that demonstrated significant changes in flow rates after exposure to higher concentrations of SO_2. This study suggests lowering the current threshold limits for SO_2 (5.0 ppm) to less than 3.0 ppm. However, results may not be statistically significant based on the small number of subjects involved in the study.

Confidence index:

Numbers of subjects in this study were not sufficient to produce statistically significant results. Furthermore, no mention of recruitment procedures was mentioned, nor were subjects informed of possible risks associated with the study. It was not clear whether subjects were randomly assigned to test or control groups. Finally, the author recorded temperature during the study, but did not record humidity nor droplet concentration of the gas mixture.

The subjects who reacted in the initial study also displayed the same symptoms later on. Although this data adds confidence to the conclusions, the effects of confounding variables was not taken into consideration. The subject selection did not exclude subjects that were asthmatic or smokers; two important variables in this type of study. While the author recognized this as a potential problem, they opted to use the data stating that the data from smokers was not significantly different from the non-smokers. This was not a valid assumption as the smokers could have been assigned to exposure groups below threshold or above saturation. The data for the asthmatics was treated separately, however, the small sample size in this group (n=2) made the findings questionable.

While the findings of the study seem to make sense, recruitment and characteristics of study subjects remain rather vague. However, the methods used in the study generally seem suitable. Also the study deals with very short exposure times, which may not be sufficient to produce more significant findings.

Review – Confidence index ranking:

Moderate to low

Author: Kulle, TJ., Sauder, LR., Shanty, F., Kerr, HD., Farrell, BP., Miller, WR and Milman, JH.	**Internal ID:** 040

Title: Sulphur Dioxide and Ammonium Sulfate Effects on Pulmonary Function and Bronchial Reactivity in Human Subjects

Year: 1984

Abstract: The effect of exposures to 1 ppm sulphur dioxide and 500 $\mu g/m^3$ respirable ammonium sulfate was studied in 20 nonsmoking subjects to determine if a response can be measured at these atmospheric levels and if the response is additive or synergistic. Four-hour separate and combined exposures were employed. Each subject acted as his or her own control and performed two light-to-moderate exercise stints for 15 minutes on each days confinement in the environmental chamber. Pulmonary function tests (body plethysmography and spirometry) and bronchial reactivity to methacholine were performed to assess the response of these exposures. No significant changes in pulmonary function or bronchial reactivity were observed in the individual exposures, the combined exposure, or 24 hours post-exposure. This study design and the observed results did not demonstrate any readily apparent risk to healthy subjects with these exposures. Since no significant changes were measured, it was not possible to conclude if these two pollutants in combination produce an additive or synergistic response.

Objective: To determine effects of sulphur dioxide and ammonium sulfate effects on pulmonary function and bronchial reactivity in human subjects.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1.0 ppm	4 hours/day, 3days/wk, 3 wks	male and female	20 to 35 years	20	healthy	1.00 ppm	unknown

Observations:

No significant changes in pulmonary function were observed with the individual exposures or the combined exposures, or 24 hour post exposure. No significant changes in pulmonary function occurred when the 10 males and 10 females were considered as separate groups. The most common symptoms were upper respiratory and eye irritation. The symptoms were most pronounced during exposure to the combination of SO_2 and $(NH_4)_2SO_4$. Seven subjects perceived the presence of sulphur dioxide gas because of its taste and/or odor and 6 subjects were aware of the combined gas/aerosol exposure.

*Statistically significant

Author's Conclusions:

"This study design and the observed results did not demonstrate any readily apparent risk to healthy subjects with these exposures. Since no significant changes were measured, it was not possible to conclude if these two pollutants in combination produce an additive or synergistic response."

Review – Assessment:

A. Subjects:	(+/-) Is the number of subjects sufficient to produce statistically significant results?

	(+) Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+/-) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+/-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+/-) Were the subjects randomly assigned to exposure groups? (+) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+/-) Were assessment measures sufficiently sensitive?

	(+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+/-) Are the results novel? (+/-) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables? (+/-) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results from this study suggest that changes in pulmonary function may have been observed if measurements had been made immediately following the first exercise. Lack of response at 4 hours could be the result of the time frame between exercise and the pulmonary function measurements or the development of tolerance over the 4-hour exposure. This study paves the way for future investigations to consider the fast response and tolerance development associated with SO_2.

Confidence index:

Because of the relatively small number of subjects involved in this study, results could not be considered statistically significant. Nevertheless, the design of the study was effective, and could be used for future studies of a similar nature. Test subjects acted as their own controls, and confounding factors were limited by accepting subjects according to pre-set criteria. While the exposure device was not explicitly described, readers were directed to a separate paper for description of the chamber. However, tolerance may have developed over the 4-hour exposure. In addition, possible synergistic effects may have occurred between SO_2 and ammonium sulfate.

Review – Confidence index ranking:

Moderate

Author: Koenig JQ. Pierson WE. Horike M. Frank R.

Internal ID: 041

Title: Effects of SO_2 plus NaCl aerosol combined with moderate exercise on pulmonary function in asthmatic adolescents.

Year: 1981

Abstract: Eight adolescent subjects with a diagnosis of extrinsic asthma and exercise induced bronchospasm were exposed for 30min at rest followed by 10min of moderate exercise on a treadmill to the following experimental modes: a) filtered air, b) 1ppm of sulphur dioxide (SO_2) and 1mg/m^3 of sodium chloride (NaCl) droplet aerosol, or c) 1mg/m^3 of NaCl droplet aerosol alone. All exposures were at >75% relative humidity and 22ºC. Functional measurements of total respiratory resistance (R_T), maximal flow at 50 and 75% expired vital capacity (Vmax50 and Vmax75), forced expiratory volume in 1 sec ($FEV_{1.0}$), and functional residual capacity (FRC) were recorded before and after exposure. Statistically significant changes were seen in R_T, Vmax50 and Vmax75, and $FEV_{1.0}$ following exposure to SO_2-NaCl droplet aerosol during exercise. The changes seen were greater than those seen in the same subjects during exposure at rest or in healthy adult subjects exposed to various air pollutants.

Objective: n/a

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1ppm	**30min @ rest, 10min @ exercise**	**6 male 2 female**	**14-18**	**8**	**Extrinsic asthmatic**	**n/a**	**n/a**

Observations:

"Statistically significant pulmonary functional changes were seen following exposure to the SO_2-NaCl droplet aerosol during exercise.

Vmax50 was reduced 44% from baseline mean ($p<0.001$) 4-5min postexercise and was still decreased by 22% ($p<0.005$) after 18min. Simultaneously, Vmax75 was reduced 50% ($p<0.005$) 4-5min postexercise, and was decreased by 90% ($p<0.050$) after 18min. R_T was increased 67% 3-4min postexercise and 64% 6-7min postexercise (not significant)."

"…we observed an average decrease in Vmax50 (-3%) and Vmax75 (-6%) after exposure to the SO_2 mixture at rest, although this time the decreases were not statistically significant."

"In contrast, exercise concurrent with the exposure produced significant changes in the partial flow-volume curves $FEV_{1.0}$ and R_T. Three subjects had shortness of breath of limited duration and five were aware of wheezing. The functional effects appear to have exceeded virtually all those changes reported for healthy exercising adults in experiments of this type, whether in response to the same concentration of SO_2-NaCl droplet aerosol (or to other pollutants)…"

*Statistically significant

Author's Conclusions:

"The functional changes following exercise appeared to involve both small airways (Vmax50 and Vmax75) and central airways ($FEV_{1.0}$ and R_T). The reduction in FEV1.0 was smaller in magnitude than that associated with the partial flow-volume curve.

Review – Assessment:

A. Subjects:	-✎ Is the number of subjects sufficient to produce statistically significant results? -✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +/-✎ Are the subjects a representative sample of the population to which extrapolations will be made? +/-✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? -✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? +✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? -✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) -✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams?

	+✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? +/-✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +/-✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +/-✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review – Summary:

Discussion of findings:

These findings demonstrate that major changes in pulmonary function and parameters can occur after exposure to low level (1ppm) SO_2 and during moderate exercise in asthmatic adolescents. Results from this study suggest that a combination of SO_2-NaCl and exercise produces changes in RT, V_{max50}, V_{max75} and $FEV_{1.0}$. However, sample size was too small to produce statistically significant results. Future studies might administer the mixture at a lower ambient temperature (22°C was used) to magnify the results seen in the current study.

Confidence index:

The study design seems adequate, although the follow-up after exposure did not continue until reversibility of symptoms occurred. Though sample size is small (n=8) and confounding factors were not specifically addressed, though each subject acted as his own control, which lends credibility to the results seen. This study did not make mention of how subjects were recruited, or if they were made aware of some of the risks associated with the study. Furthermore, confounding factors such as smoking or previous medical conditions unrelated to asthma were not investigated. The number of subjects involved in the study (8) was not sufficient to allow for statistically significant results. The study also alludes to a SO_2-NaCl monitoring device, but a full description of the device is available in another paper.

Review – Confidence index ranking:

Moderate to low

Author: Koenig, JQ., Pierson, WE., Horike, M. and Frank, R.	**Internal ID:** 042
Title: Effects of Inhaled Sulphur Dioxide on Pulmonary Function in Healthy Adolescents: Exposure to SO_2 Alone or SO_2 + Sodium Chloride Droplet Aerosol During Rest and Exercise	
Year: 1982	
Abstract: Recently, we reported statistically significant changes in pulmonary functional measurements in asthmatic adolescents exposed at rest or during exercise. To demonstrate whether those results were due to the subjects' adolescence, or more likely, to their diagnosis of asthma, we repeated identical exposures in healthy adolescents. The healthy subjects showed small, statistically significant changes after exposure to sulphur dioxide; however the changes were only slightly greater than those seen after sham exposure. After exposure at rest to 1.0 ppm SO_2 + 1mg/m^3 sodium chloride (NaCl) droplet aerosol, the healthy adolescents showed small (3%) but statistically significant reductions in forced expiratory volume in one second ($FEV_{1.0}$). No significant changes were seen following exposure to NaCl or SO_2 alone at rest. Exposure to the SO_2 modes during moderate exercise produced greater changes. Following exposure to either the SO_2 + NaCl mixture or 1.0 ppm of SO_2 alone, the reductions in $FEV_{1.0}$ were slightly greater (6%) and prolonged compared with those seen at rest. Significant decreases also were seen in V_{max50} and V_{max75}. A small decrease (4%) in $FEV_{1.0}$ was seen following exposure to NaCl alone during exercise, but the change was not statistically significant. The changes seen in the healthy adolescents were slight compared to those seen in the asthmatic adolescents previously exposed (changes of 23-67%). We conclude that asthmatic adolescents are much more sensitive to the effects of inhaled SO_2 than are healthy adolescents.	
Objective: To determine the effects of inhaled sulphur dioxide on pulmonary function in healthy adolescents during rest and exercise.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1.0 ppm	40 min	male and female	12-14 years	8	healthy	unknown	unknown

Observations:

The mean decrease in FEV_1 from baseline following 30 min of exposure at rest to 1ppm SO_2 + 1.0mg/m^3 NaCl droplet aerosol was statistically significant ($p<.025$) .There were no statistically significant changes pulmonary function following 30 min of exposure at rest to the NaCl droplet aerosol alone or SO_2 alone. The mean decrease in FEV_1 from baseline 2-3 min after exposure to SO_2 alone during exercise was statistically significant ($p<.001$).The only other point when the mean decrease was statistically significant was at 7-8 min post-exposure ($p<.05$). The decreases in max flow at 50% and 75% vital capacity were significant after 7-8 min. Decreases in FEV_1 2-3min and 4-5min after exposure to the mixture were significant. The decrease in max flow at 50% vital capacity was stat. significant 4-5 min post exercise. Increases in functional residual capacity were statistically significant There were no consistent responses on the symptom rating scale and no report of subjective symptoms during or after any of the tree expose modes.

*Statistically significant

Author's Conclusions:

"We conclude that asthmatic adolescents are much more sensitive to the effects of inhaled SO_2 than are healthy adolescents."

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+/-) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+/-) Were the subjects randomly assigned to exposure groups? (+) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive?

	(+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Findings from this paper indicated that asthmatic adolescents are much more sensitive to the effects of inhaled SO_2 than are healthy adolescents. While this study was the first of its kind to report acute effects of inhaled SO_2 in a group of carefully characterized healthy adolescents, the number of people involved in the study (8) prevents results from being considered statistically significant and findings extrapolated. Despite this, results support the hypothesis that asthmatic adolescents are more sensitive to inhaled effects of an air pollutant than adolescents who do not have asthma or other allergic diseases. In addition, no significant changes were seen following exposure to NaCl or SO_2 alone at rest. This may indicate possible synergistic effects or errors in the measurements and calculations.

Confidence index:

Prior to the study, investigators completed extensive medical evaluations on all of the subjects, along with performed a series of laboratory tests. While the method of recruitment was not outlined in the study, all subjects were made aware of the risks associated with the subject, and consent forms obtained. Furthermore, the author described the reason for the recruitees who withdrew from the study. The author further describes the technique used to randomly distribute subjects among testing regimes. For exposure at rest, the mixture produced some significant effects, but the single exposures at rest did not. This indicates possible errors in the measurements or analysis, or synergistic effects.

Review – Confidence index ranking:

Moderate

Author: Stacy RW. Seal E Jr. House DE. Green J. Roger LJ. Raggio L.	**Internal ID: 043**

Title: A survey of effects of gaseous and aerosol pollutants on pulmonary function of normal males.

Year: 1981

Abstract: A total of 231 normal male human subjects were exposed for 4 hr to air, ozone, nitrogen dioxide, or sulphur dioxide; to sulfuric acid, ammonium bisulfate, ammonium sulfate, or ammonium nitrate aerosols; or to mixtures of these gaseous and aerosol pollutants. Only one concentration of each pollutant was used. This study, therefore, represents a preliminary survey, intended to allow direct comparison of studies to plan future research. During exposure each subject had two 15-min exercise sessions on a treadmill at 4 mph and 10% grade. Environmental conditions were mildly stressful, i.e., temperature = 30 degrees C and relative humidity = 60%. A battery of 19 measurements of pulmonary function was performed just prior to exposure (air control); 2 hr into the exposure, following the first exercise session; 4 hr into the exposure, following the second exercise session; and 24 hr after exposure. Significant differences were noted in specific airway resistance (SRAW), forced vital capacity (FVC), and forced expiratory flow at 50% of FVC (FEF50) and in related measurements in those experimental groups exposed to ozone or to ozone plus aerosols. None of the aerosols alone, nitrogen dioxide or sulphur dioxide alone, or mixtures of nitrogen dioxide or sulphur dioxide with aerosols produced significant effects. A distribution analysis of subject responsivity to ozone gave a normal distribution among subjects not exposed to ozone, and a distribution shifted to the right and skewed to the right among those exposed to ozone alone or in mixture, with no evidence of bimodal distribution of ozone sensitivity.

Objective: To determine the effects of a single concentration of gaseous and aerosol pollutants on pulmonary function of normal males.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.75ppm	**6 times each - 4 hours**	**Male**	**18-40**	**231**	**Healthy nonsmoking**	**n/a**	**n/a**

Observations:

Significant differences were noted in specific airway resistance (SRAW), forced vital capacity (FVC), and forced expiratory flow at 50% of FVC and in related measurements in those experimental groups exposed to ozone or to ozone plus aerosols. None of the aerosols alone, nitrogen dioxide or sulphur dioxide alone, or mixtures of nitrogen dioxide or sulphur dioxide with aerosols produced significant effects. A distribution analysis of subject responsivity to ozone gave a normal distribution among subjects not exposed to ozone, and a distribution shifted to the right and skewed to the right among those exposed to ozone alone or in mixture, with no evidence of bimodal distribution of ozone sensitivity.

*Statistically significant

Author's Conclusions:

"This experiment demonstrates a method of studying a number of pollutants and pollutant mixtures with identical, and therefore comparable, procedures. Using some form of this protocol, it will be possible to extend the studies to other pollutants and pollutant mixtures, to several different pollution groups, and to several other concentrations of pollutants. The use of a "standard protocol" would render the comparison of experiments published in the literature feasible."

Review – Assessment

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +✎ Are the subjects a representative sample of the population to which extrapolations will be made? +/-✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +/-✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? +/-✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+/-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? +✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale?

	+✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? +✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✏ Was individual data reported for each subject? -✏ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✏ Are the statistical methods used appropriate? -✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
G. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective? +✏ Were assumptions reasonable? +✏ Are the methods reproducible? +✏ Did either the study design or the statistical analysis limit confounding variables? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Although the only significant findings of respiratory effects in this study come from ozone, the lack of significance with the wide range of other pollutants studied is likely only due to the low concentrations being tested. While results from this study can be considered statistically significant, no gradient of exposures was used. Nevertheless, results of this experiment may be used to simplify future experimental protocols.

Confidence index:

The design of this study is very elegant, with a 4x5 factorial analysis of combinations of various air pollutants. Exposure durations are long (4hours), and the methods and statistics used all appear to be of very high quality. Furthermore, the study has a large sample size of persons with characteristics well recorded. The large sample pool is a little misleading as they needed to be split among 19 test groups. The experimental design was novel, however, the study was too ambitious. The large sample pool could have been used in a more effect way; possibly a study of 2 gases at different concentrations. Also, the concentrations of the gases were probably below threshold especially with the employed delivery system. While the study alludes to description of the exposure chambers in other documents, it does not include exposure chamber specifics in the document. Furthermore, no mention of how the subjects were recruited or if they were informed of the risks of the investigation was given.

Review – Confidence index ranking:

High to moderate

Author: Newhouse MT. Wolff RK. Dolovich M. Obminski G.	**Internal ID: 045**
Title: Effect of TLV levels of SO_2 and H_2SO^4 on bronchial clearance in exercising man.	
Year: 1978	
Abstract: Pulmonary mucociliary function was assessed following exposure to industrial threshold limit values (TLV) of sulphur dioxide (5 ppm) SO_2) and sulphuric acid mist (1 mg/m^3 H_2SO_4). Bronchial clearance was measured in two sets of ten healthy exercising non-smoking adults under control and exposure conditions. A 99mTc-albumen saline aerosol (MMD 3 micrometer) was inhaled as a bolus in late inspiration under controlled conditions to produce reproducible deposition in large airways. Lung retention of radioactivity was quantified using a gamma camera and computer analysis. Clearance was significantly faster (P less than .05) on exposure to both SO_2 and H_2SO_4 compared to control values. Maximum mid-expiratory flow rates (MMFR) were significantly reduced (P less than .01) on exposure to SO_2 (mean decrease 8.5%), but only slightly reduced for H_2SO_4 (1.4%). The speeding in clearance was probably an irritant response in both cases. For SO_2 the response appeared predominantly reflex, while H_2SO_4 showed evidence of a direct effect.	
Objective: "The present study describes the acute effect of TLV (Threshold Limit Values) of SO_2 and H_2SO_4 on bronchial clearance associated with a vigorous exercise period, comparable to an industrial work situation."	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
5ppm	**2.5 hours**	**Male+Female**	**19-34**	**10**	**healthy**	**n/a**	**n/a**

Observations:

At 2 hours exposure to SO_2, there were no significant changes in VC or FEV1.0. However "…there were changes in maximum midexpiratory flow rate (MMFR). Exposure to SO_2 produced a highly significant mean decrease of 8.5% ($p<0.01$)…" "…if retention after 2 hours differed by more than this amount (SD of the mean difference percentage retention 6.7%) from the control experiments, the changed was deemed marked. Six of the ten subjects showed a markedly increased clearance on SO_2 exposure. One subject showed a markedly decreased clearance, and three subject showed little change."
*Statistically significant

Author's Conclusions:

Clearance was significantly faster on exposure to both SO_2 and H_2SO_4 compared to control values. Maximum mid-expiratory flow rates were significantly reduced on exposure to SO_2 but only slightly reduced for H_2SO_4. The speeding in clearance was probably an irritant response in both cases. For SO_2 the response appeared predominantly reflex, while H_2SO_4 showed evidence of a direct effect.

Review – Assessment

A. Subjects:	+✓ Is the number of subjects sufficient to produce statistically significant results? +/-✓ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate

	for the objective of the study? +/-✎ Are the subjects a representative sample of the population to which extrapolations will be made? +/-✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +/-✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	+/-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? +/-✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+/-✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✎ Was individual data reported for each subject?

	+/-✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +/-✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Clearance of mucus from the lungs was found to increase significantly, and MMEF was found to decrease significantly upon exposure to 5.0ppm SO_2. While the results are statistically significant, varying individual responses were shown. Of the 10 study subjects, 3 showed no effect in terms of clearance, while one subject actually decreased. However, due to a small sample size, results cannot be considered statistically significant. Nevetheless, results suggest that exercise speeds the rate of clearance from the lung. However, the author also points out that mucociliary transport impairment might occur following longer exposures, or in people with underlying lung disease.

Confidence index:

Although the results are significant, individual variation was demonstrated in response to SO_2 exposure. The small sample size (n=10), skewed sex ratio (9M:1F) and individual variation adds some uncertainty to the findings, though they are what one would logically expect. However, the methods and conduct of the study seem appropriate for the objective. The use of a radioactive detection method was surprising from a subject health standpoint and a confounder standpoint. Although the concentrations are low, the health effects of the radioactive agent are unknown and could act as a synergistic agent in conjunction with SO_2 in examining the endpoint. It is unknown if the subjects were smokers, on medications, atopic, etc. Furthermore, subject recruitment procedures or description of study risks and benefits is not included in the material and methods section.

Review – Confidence index ranking:

Moderate to low

Author: Carson, JL., Collier, AM., Hu, S., Smith, CA. and Stewart, P.	**Internal ID:** 046
Title: The Appearance of Compound Cilia in the Nasal Mucosa of Normal Human Subjects Following Acute, in Vivo Exposure to Sulphur Dioxide	
Year: 1985	
Abstract: Electron microscopic examination of ultrathin sections of ciliated nasal epithelium obtained from seven normal, healthy human volunteers indicated increases in the prevalence of compound cilia following controlled, acute exposure to 0.75 ppm sulphur dioxide (SO_2). Morphometric analyses of the specimens confirmed that a statistically significant association between SO_2 exposure and compounding of nasal epithelial cilia in four of the seven subjects. Concomitant freeze-fracture replicas prepared from these samples also revealed the occurrence of compound cilia with accompanying evidence of abnormal ciliary membrane ultrastructure in the nasal epithelium. These studies indicate that SO_2 may be implicated as a causative agent in ciliary compounding in the upper respiratory tract and hat compound cilia represent a form of acquired ciliary defect that may serve as a readily quantifiable marker of epithelial injury.	
Objective: To characterize ultrastructural features of the nasal mucosal of human volunteers following acute, in vivo SO_2 exposure. Particular attention was given to patterns of epithelial cell injury that might be associated with small or undetectable decrements in mucociliary clearance.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.75 ppm	2 hours	unknown	unknown	7	healthy	unknown	unknown

Observations:

Screening of ultrathin sections of post exposure samples of nasal epithelium revealed an apparent increase in the prevalence of compound cilia in several individuals. Three subjects elicited no positive correlative response to SO_2 exposure relative to the appearance of compound cilia and of these, one individual exhibited no compound cilia in either pre or postexposure samples. Morphometric analysis examining the frequency of ciliary compounding confirmed statistically significant increases in the prevalence of compound cilia following SO_2 exposure in four of the seven subjects. Tests for the association between SO_2 exposure and ciliary compounding also yielded a combined p value for the seven subjects of $p<0.0001$.

*Statistically significant

Author's Conclusions:

"These studies indicate that SO_2 may be implicated as a causative agent in ciliary compounding in the upper respiratory tract and that compound cilia represent a form of acquired ciliary defect that may serve as a readily quantifiable marker of epithelial injury."

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?

	(+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+/-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) Was the mode of administration appropriate? (+/-) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+/-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+/-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Were the subjects randomly assigned to exposure groups? N/A Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+/-) Was the length of follow-up appropriate? (+/-) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+/-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject?

	(+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+/-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results from this study suggest that SO_2 may be implicated as a causative agent in ciliary compounding in the upper respiratory tract. This is the first study of its kind to deal specifically with the motility or functional impairment of mucociliary clearance. This study, although without statistically significant results, paves the way for more research into functional decrements conferred on mucociliary transport by SO_2.

Confidence index:

This paper uses a novel method of exploring physiological damage from exposure from SO_2 exposure. The figures in the paper show marked changes in cilia morphology in the "before and after" pictures for SO_2 exposure. Results are consistent with findings from other studies. However, it is likely the authors chose to use the best data in their study. From a clinical point a view, this study gives some evidence of the injury that long exposures of low levels of SO_2 cause. However, there are a number of issues involved. The criteria for compound cilia is subjective one and requires highly trained personnel. Pre-existing conditions such as nasal infections, allergies, nasal medications and smoking could make subjects more prone to this endpoint with SO_2 exposure. Without the referenced paper, one cannot fully evaluate this possibility. No control group was incorporated into the study and the number of subjects (7) is inadequate to produce statistically significant results. In addition, only one concentration of SO_2 was used. Furthermore no description of the sensitivity or the monitoring device, or even a detailed description of the device is given.

Review – Confidence index ranking:

Low to moderate

Author: Bedi JF. Folinsbee LJ. Horvath SM.	**Internal ID:047**
Title: Pulmonary function effects of 1.0 and 2.0 ppm sulphur dioxide exposure in active young male non-smokers.	
Year: 1984	
Abstract: The threshold concentration of sulphur dioxide which will induce pulmonary function changes in the normal population has not been established. To investigate this question, nine young healthy adult non-smoking males were exposed for 2hrs to filtered air (FA), 1.0ppm SO_2, and 2.0ppm SO_2. In a follow-up study an additional 14 subjects were exposed to FA and 1.0ppm SO_2 conditions. Forced expiratory tests were preformed prior to and following the 2hr exposure, and 5min after each of the three 30-min exercise periods. The work load during the exercise periods was set to elicit a minute ventilation of 40 liters BTPS. Thoracic gas volume (TGV), airway resistance (Raw), maximal voluntary ventilation (MVV), and helium dilution functional residual capacity (FRC) were also measured before and after the exposure. In the initial subject group (n=9), none of the spirometric tests demonstrated any significant effects to the subject from exposure to 1.0 or 2.0ppm SO_2 either after 30min of continuous exercise or 2 hrs of intermittent exercise. The only significant change ($p<0.05$) was an increase in specific airway resistance (SRaw) following exposure to 1.0 or 2.0ppm SO_2. However, when this observation was further studied in the larger (n=23) subject group exposed to 1.0ppm SO_2, neither SRaw nor any of the other pulmonary function tests indicated a significant effect of exposure to 1.0ppm SO_2.	
Objective: NA	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1.0-2.0ppm	**Single exposure 2hrs**	**Male**	**19-28**	**9 + 14 = 23**	**Healthy nonsmoking**	**n/a**	**1.0ppm**

Observations:

At rest: "Inspiratory capacity decreased significantly following the 2ppm SO_2 exposure and was significantly ($p<0.045$) less than the post exposure value at 1.0ppm SO_2."

After exercise: "In summary, none of the flow of volume measures demonstrated any significant effects from exposure to either 1.0 or 2.0ppm SO_2 after ½ h of continuous exercise or 2 hour of intermittent exercise."

"Airway resistance increased ($p<0.02$) following exposure to 1.0 or 2.0ppm SO_2, but not following FA (filtered air) exposure."

*Statistically significant

Author's Conclusions:

"In summary, it appears that exposure to 1.0ppm SO_2 in normal subjects can cause changes in airway resistance. The significance of such changes is dependent on a number of factors, including individual subject sensitivity, duration of exposure, intensity and duration of activity during exposure, and the route of SO_2 inhalation. However, alterations in forced expiratory measures are not likely to occur at 1.0ppm SO_2."

Review – Assessment

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +/-✎ Are the subjects a representative sample of the population to which extrapolations will be made? +✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? +✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +/-✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? +/-✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical?

	+✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? +/-✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? +-✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +/-✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The only effects seen from exposure to 1.0 and 2.0ppm SO_2 exposures with exercise were increased airway resistance, while functional changes in respiratory capacity were only seen following exposures of 2.0ppm SO_2. While some changes can be noted from 1.0ppm exposures, responses are likely to range between individuals. Though the responses in this study seem to be minimal, they nonetheless demonstrate that even low-level SO_2 exposures can affect healthy persons. Furthermore, results appear to be in striking contrast with those observed in asthmatics. Nevertheless, the small sample size prevents statistical significance from being attached to the results.

Confidence index:

The methods used to collect and analyze data in this study appear to be rigorous, and the sample size (n=23) is larger than most clinical studies. This study ensured explanation of procedures and risks to subjects, and sought written consent. However, no mention of recruitment tactics was given, or if any subjects withdrew. However, investigators limited confounding factors by giving a medical history questionnaire plus a physical examination to each subject prior to the study. In general, experimental design well thought out, all exposure and monitoring equipment was explicitly detailed. More information about individual subjects would have been helpful. The statistical level of significance was set very conservatively, and thus more end-points may have been significant at the $p<0.01$ level. The authors refuted the findings in other papers due to following reasons:

-Incorrect statistical interpretation
-Confounders
-Timing of measurements
-Route of exposure

Review – Confidence index ranking:

Moderate to high

Author: Andersen, IB., Jensen, PL., Reed, SE., Craig, JW., Proctor, DF. and Adams, GK.

Internal ID: 048

Title: Induced Rhinovirus Infection Under Controlled Exposure to Sulphur Dioxide

Year: 1977

Abstract: The interaction between short-term sulphur dioxide exposure and experimentally induced rhinovirus infection was studied in thirty-two volunteers divided into two groups balanced with respect to age, antibody levels, and nasal mucus flow rates. One group was exposed to SO_2 at the threshold limit value (TLV) of 5 ppm during 4 hours; the other group served as controls exposed to pollution–free air under the same conditions. The SO_2 exposure caused a 50% decrease in nasal mucus flow rate in the anterior parts of the nose, but there was no difference in the number of colds which developed in the two groups. The group exposed to SO_2 had fewer symptoms an a possibly shorter incubation period, and virus shedding was at a lower level but more persistent than in the control group. No differences were founding antibody response. The rhinovirus infection in the control group caused a gradual decrease in nasal mucus flow rate starting 2 days after the virus instillation, and after 5 days the rate was less than half its initial value. For future experiments on the interaction between airborne pollutants and rhinovirus infections, a virus challenge by aerosol inhalation is recommended. Our study supports an earlier observation that growth of influenza virus in the nasal cavity of mice was inhibited by exposure to SO_2 concentrations of 6 or 20 ppm.

Objective: To learn if SO_2 exposure at the TLV affects susceptibility to and severity of artificially induced rhinovirus infection, and to gain further information on the effect of experimental colds on mucociliary flow.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
5 ppm	4 hours	male and female	17 to 47	32	healthy	unknown	5 ppm

Observations:

In the SO_2 exposure group thirteen subjects received rhinovirus and ten infection s developed, whereas nine out of twelve subjects in the control group became infected, an insignificant difference. None of the seven subjects that were exposed to saline instead of the rhinovirus developed a cold. In those subjects with naturally fast clearance, fifteen of twenty virus-exposed subjects were infected, while of those with naturally slow clearance four of five became infected – not a significant difference. The SO_2 exposure group had fewer symptoms than the control group. The average incubation period was 55 hours in group A(Exposure/Rhinovirus/Infection) and 63 hours in group B (no exposure/Rhinovirus/Infection) with ranges from 24-72 hours and from 60-84 hours respectively $P=.06$). ($P<.05$) Virus was never recovered from the uninfected group.Virus shedding was at a lower level but more persistent in the SO_2 exposed group. The differences in the first trial between the exposed and nonexposed groups was statistically significant except on day 7. No differences were found in antibody response. The rhinovirus infection in the control group caused a gradual decrease in nasal mucus flow rate starting 2 days after the virus instillation, and after 5 days the rate was less than half its initial value.

*Statistically significant

Author's Conclusions:

"I n our experiment, artificially induced slowing of nasal mucociliary flow rate by SO_2 exposure failed to influence the attack rate for the common cold, as induced by instillation of rhinovirus. Furthermore, symptoms associated with colds were less severe in subjects exposed to SO_2. These findings may reflect an SO_2 effect on the host, the rhinovirus, or both. Our study supports an earlier observation that growth of influenza virus in the nasal cavity of mice was inhibited by exposure to SO_2 concentrations of 6 or 20 ppm."

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+/-) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? (+/-) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+) Was the type of blinding indicated? (*i.e.,* single, double, triple, etc)

	(+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+/-) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+/-) Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results from this study suggest that introduction of SO_2 into the mucociliary tract failed to influence the attack rate of the common cold. These results are in conflict with previous studies that indicate that increased concentrations of SO_2 in the air are associated with an increase in respiratory infection. Results further indicated that symptoms seemed less severe in subjects exposed to SO_2, suggesting that SO_2 may directly affect the virus. Future studies might consider examining the effects of a gradient of SO_2 exposure concentrations on the mucociliary tract.

Confidence index:

The discussion section of this paper makes mention of only two other studies. This study does not make mention of how subjects were recruited into the study, or if any chose to withdrawal. Furthermore, it is not clear whether subjects were made of aware of the risks or benefits of the study. The volunteers were housed in isolation flats and allowed to mix with each other. In addition, it is unclear whether subjects were randomly assigned to study groups, and, if so, how the randomization was achieved. Only one exposure level of SO_2 was administered 2 of the seven days at 4.5 hours each. Duration may not have been long enough to elicit response. The high exposure concentration used was not representative of ambient concentrations. Furthermore, subjects were at rest during exposure, whereas results may have differed during normal daily exercise. Instillation of the fluid containing rhinovirus was carried out with the subject lying supine and the head hanging downward. The region of nasal mucociliary defences impaired by SO_2 exposure may not have been the same region that was initially exposed to the virus – this was pointed out by the authors.

Review – Confidence index ranking:

Moderate to low

Author: Bedi JF. Horvath SM. Folinsbee LJ. **Internal ID: 049**

Title: Human exposure to sulphur dioxide and ozone in a high temperature-humidity environment.

Year: 1982

Abstract: In order to examine the role of high temperature and humidity in the possible synergistic action of ozone and sulphur dioxide on human pulmonary function previously reported by some investigators and repudiated by others, we randomly exposed eight healthy young male nonsmokers to filtered air, 0.4 ppm SO_2, 0.4 ppm O_3, and 0.4 ppm SO_2 plus 0.4 ppm O_3 at 35 degrees C--85% rh for 2 hours. Subjects exercised for 15 minutes of each half hour at a workload sufficient to elicit a minute ventilation of 30 liters (BTPS). Pulmonary function tests were performed prior to, during, and following exposure. Observed alterations in pulmonary functions in the $SO_2 + O_3$ exposure reflected the changes occurring during the O_3 exposure, clearly indicating that no synergistic effects related to the additional presence of SO_2 were evident in this study. We considered factors suggested as causes for a potential synergism in comparing the conflicting studies on O_3 and SO_2, and suggest that factors other than temperature-humidity and/or sulfate aerosol production must be sought.

Objective: n/a

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.4ppm	**2hrs**	**Male**	**19-32**	**8**	**Healthy**	**0.4ppm**	**n/a**

Observations:

"No functional changes in $FEV_{1.0}$ occurred as a result of exposure to filtered air or 0.4ppm SO_2, but significant decrease occurred following exposure to either 0.4ppm O_3 (6.9%) or the combination of 0.4ppm O_3 + 0.4ppm SO_2 (7.4)%. However, no significant differences were found between the ozone exposure and the ozone + sulphur dioxide exposure."

For none of the lung function parameters were differences noted between O_3 and $O_3 + SO_2$ exposures, suggesting no synergistic effects.

*Statistically significant

Author's Conclusions:

"The major hypothesis examined in this study was that the ozone-sulphur dioxide synergism which had been demonstrated in some studies, and not others would occur if a high water vapor content would be present in the ambient environment. The hypothesis was not confirmed since no pulmonary function measurements demonstrated differences between ozone exposure alone and the ozone plus sulphur dioxide condition."

Review – Assessment:

A. Subjects:	-✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? -✎ Are the subjects a representative sample of the population to which extrapolations will be made?

	+/-✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical?

	+✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? +/-✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? -✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:.

This study used 8 young male subjects. The age, weight and height were reported but there was no medical history. The results of the study show that there is no significant synergistic effect between O_3 and SO_2 in a humid environment. This result is not surprising considering the low concentrations used for SO_2 (0.4 ppm) and the method of exposure (chamber not direct exposure with mouthpiece). The authors argue that there are overall decreases in a number of pulmonary endpoints (FVC and IC) and increases in others (FRC, RV and Raw), they are mostly statistically insignificant according to the data.

Confidence index:

The experimental design was standard for this type of study. Experimental procedures were well described in the text and the exposure and measurement intervals were logical. The lack of information about the subjects raises the issue of confounders as smokers, subjects on medication, asthmatics would surely skew results of the study. Lastly, the SO_2 concentration was very low for this type of study and it was not surprising that no synergistic effect was found between O_3 and SO_2 at these levels.

Review – Confidence index ranking:

Moderate to high

Author: Bedi JF. Folinsbee LJ. Horvath SM. Ebenstein RS.	**Internal ID: 051**
Title: Human exposure to sulphur dioxide and ozone: absence of a synergistic effect.	
Year: 1979	
Abstract: Studies of the human health effects of exposure to a combination of ozone and sulphur dioxide have produced somewhat conflicting results; the possibility of a synergistic enhancement of toxicity when the two gases are present simultaneously remains equivocal. We evaluated the effects of 0.40 ppm sulphur dioxide, 0.40 ppm ozone, and the combination of these two under one environmental condition (25 degrees C and 45% relative humidity). Subjects alternately walked and rested during a 2-hr exposure. Subjects exposed to filtered air or to 0.40 ppm sulphur dioxide showed no significant changes in pulmonary function. When exposed to either ozone or ozone plus sulphur dioxide, significant decreases in maximum expiratory flow, forced vital capacity, and inspiratory capacity were observed. There were no significant differences in response between ozone alone and ozone plus sulphur dioxide exposures, thus, in our subjects on synergistic effects were discernible.	
Objective: "The major hypothesis to be tested was that of O_3-SO_2 synergism; specifically, does the addition of low concentrations of SO2 to the atmosphere enhance the known effects of SO2 on the respiratory system?"	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.4ppm	**2hours**	**Male**	**18-27**	**9**	**Healthy**	**n/a**	**n/a**

Observations:

No statistically significant changes in respiratory function were noticed following exposure to SO_2 alone, nor were synergistic effects noted when subjects were exposed to ozone and SO_2 concurrently. All respiratory effects seen in the study appear to be the result of exposure to ozone.
*Statistically significant

Author's Conclusions:

"There were no overall trends in pulmonary function data which indicated any difference between the exposure to O_3 alone and the exposure to O_3+SO_2. Under the conditions of this experiment, the concentration of SO_2 used in this study was insufficient to provoke any pulmonary function changes, and it caused no additional pulmonary function alterations to those produced by O_3 alone."

Review – Assessment

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ±✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an

	unrepresentative sample population? +✏ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	+✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✏ Was there a gradient of exposure levels? +✏ Were the chosen exposure levels appropriate to investigate the primary objective? +✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? +✏ Was evidence provided to justify the dosing regime? +✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✏ Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✏ Were the subjects randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? -✏ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✏ Was the length of follow-up appropriate? +✏ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? -✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✏ Was individual data reported for each subject? ±✏ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✏ Are the statistical methods used appropriate? +✏ Are confidence intervals reported?

	+✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? -✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study found no synergism between concurrent exposures to ozone and SO_2 in terms of respiratory changes. However, SO_2 exposure did not effect any changes in respiratory function, even alone, which is likely owing to the low concentrations (0.4ppm) used in this study. For this reason, the possible synergism of SO_2 and ozone exposures cannot be completely dismissed, as it may occur at higher exposure levels. This result disputes a study by Hazucha and Bates who found a 30 to 50% decrease in various measures of maximum expiratory flow when 0.37 ppm and 0.37 ppm SO_2 were administered in combination. However, results from the current study are in keeping with the majority of research that has been done on this topic. Future studies of this kind should include more than nine subjects in order to ensure results that are statistically significant.

Confidence index:

Although sample size is small (n=9), the study appears to have been conducted in a satisfactory manner. However, the study cannot rule out a possible synergism at higher exposure levels. It is puzzling why the authors chose to use subjects that were not all healthy or all "sensitive". The mixture of the types of subjects would give rise to confounders. In this case however, the ends justify the means as no significant effects were found for SO_2 exposure. This is probably due to the low concentration of SO_2 that is likely lower than the threshold even for sensitive individuals.

Review – Confidence index ranking:

Moderate to high

Author: Field, PI., Simmul, R., Bell, SC., Allen, DH. And Berend, N. **Internal ID:** 052

Title: Evidence for Opioid Modulation and Generation of Prostaglandins in Sulphur Dioxide-Induced Bronchoconstriction

Year: 1996

Abstract: Inhalation of sulphur dioxide provokes bronchoconstriction in asthmatic subjects. Cholinergic mechanisms contribute, but other mechanisms remain undefined. The effect of morphine, an opioid agonist, on the cholinergic component of SO_2-induced bronchoconstriction was investigated, and the effect of indomethacin, a cyclooxygenase inhibitor, on SO_2-induced bronchoconstriction and tachyphylaxis was studied. In the first study 16 asthmatic subjects inhaled either ipratropium bromide or placebo 60 minutes before an SO_2 challenge on days 1 and 2. On day 3 an SO_2 challenge was performed immediately after intravenous morphine. In the second study 15 asthmatic subjects took either placebo or indometacin for three days before each study day when two SO_2 challenges were performed 30 minutes apart. The response was measured as the cumulative dose causing a 35% fall in specific airways conductance. Ipratropium bromide significantly inhibited SO_2 responsiveness, reducing PdsGaw by 0.89 doubling doses. This effect persisted after correction for bronchodilation induced by ipratropium bromide. The effect of ipratropium bromide and morphine on SO_2 responsiveness also correlated. In the second study SO_2 tachyphylaxis developed with PdsGaw on repeated testing, being reduced by 0.62 doubling doses. Indomethacin attenuated baseline SO_2 responsiveness, increasing PdsGaw by 0.5 doubling doses. These results suggest that opioids modulate the cholinergic component of SO_2 responsiveness and that cycoloxygenase products contribute to the imeediate response to SO_2.

Objective: The effect of morphine, an opioid agonist, on the cholinergic component of SO_2-induced bronchoconstriction was investigated, and the effect of indomethacin, a cyclooxygenase inhibitor, on SO_2-induced bronchoconstriction and tachyphylaxis was studied.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.5 to 8.0 ppm	3 visits	Male and female	19-60	31	Mild asthma	unknown	unknown

Observations:

In the first study 16 asthmatic subjects inhaled either ipratropium bromide or placebo 60 minutes before an SO_2 challenge on days 1 and 2. On day 3 an SO_2 challenge was performed immediately after intravenous morphine. In the second study 15 asthmatic subjects took either placebo or indometacin for three days before each study day when two SO_2 challenges were performed 30 minutes apart. The response was measured as the cumulative dose causing a 35% fall in specific airways conductance. Ipratropium bromide significantly inhibited SO_2 responsiveness, reducing PdsGaw by 0.89 doubling doses. This effect persisted after correction for bronchodilation induced by ipratropium bromide. The effect of ipratropium bromide and morphine on SO_2 responsiveness also correlated. In the second study SO_2 tachyphylaxis developed with PdsGaw on repeated testing, being reduced by 0.62 doubling doses. Indomethacin attenuated baseline SO_2 responsiveness, increasing PdsGaw by 0.5 doubling doses.

*Statistically significant

Author's Conclusions:

"These results suggest that opioids modulate the cholinergic component of SO_2 responsiveness and that cycoloxygenase products contribute to the imeediate response to SO_2."

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+/-) Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+/-) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+) Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom)

	(+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+/-) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+/-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The objective of this study was to obtain information on the mechanism of bronchialconstriction due to SO_2 exposure. Results from this study indicated that opioids modulate the cholinergic component of SO_2 responsiveness and that cyclooxygenase products contribute to the immediate response to SO_2. Furthermore, this study reported mechanisms that could contribute to the response of the airways to SO_2 in subjects with asthma. Ipratropium bromide provided a variable protection against inhaled SO_2. There was no relationship between the degree of protection afforded by ipratropium bromide and the degree of bronchodilatation. The results imply that opioids modulate the cholinergically mediated component of the bronchoconstrictive response to SO_2. However, further research is needed before this can be confirmed. The attenuation of initial SO_2 responsiveness by indomethacin suggests that the airway response to SO_2 is partially mediated by cyclooxygenase products present in the airways of asthmatic subjects. After indomethacin the mean change in SO_2 responsiveness between the first and second challenge was significantly less than after placebo. Therefore it is difficult to determine whether this change in SO_2 responsiveness is due to the indomethacin or if the indomethacin inhibited the development of SO_2 tachyphylaxis by indomethacin. This study contributes to previous research that associated sulphur dioxide with bronchoconstriction in asthmatics. Furthermore, according to the author, there was only one other published study demonstrating opioid inhibition of cholinergically mediated bronchoconstriction in humans *in vivo*. Consequently, this study provides useful, and statistically significant data that can be applied to similar situations.

Confidence index:

This study utilized a moderate level of subjects (31). The author did not make mention of how subjects were recruited or if they were aware of experimental risks. However, confounders such as age, gender, smoking, and respiratory history were addressed. This study makes use of a gradient of exposures but does not explain how dosages were determined. Furthermore, no information about naloxone was given. Five of the subjects were also taking inhaled steroids, which could reduce their sensitivity to the exposures in this study, although they were asked to withhold medication for six hours or more before testing. Finally, only nominal exposure concentrations were recorded, potentially incorporating error into the study results.

Review – Confidence index ranking:

Moderate

Author: Toyama T, Nakamura K	**Internal ID: 053**

Title: Synergistic response of hydrogen peroxide aerosols and sulphur dioxide to pulmonary airway resistance.

Year: 1964

Abstract: In 24 healthy volunteer males, changes of pulmonary airway resistance were measured in the following sequence of inhalation; 1) after short term inhalation of hydrogen peroxide aerosols in two groups of 4.6μ and 1.8μ of their count median diameter, 2) sulphur dioxide (1-60 ppm), 3) mixture aerosols of hydrogen peroxide and sulphur dioxide, 4) bronchodilator, 5) the mixture aerosols of the same to 3), and their effects were compared with the pre-experimental control level. A synergistic increase of the response was observed by the mixture aerosols which were recognized as sulfuric acid mist. Difference of the particle size resulted in different response to the respiratory tracts.

Objective: The present paper deals with the response of pulmonary airway resistance in man, comparing the toxicity between sulphur dioxide and its converted sulfuric acid mist

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1-60ppm	**5min**	**Male**	**20-28**	**24**	**healthy**	**n/a**	**n/a**

Observations:

"The inhalation of sulphur dioxide of 1-60ppm alone resulted in the increase of the airway resistance; in the first group mean percentage change was 19.6% higher than the control value and 11.3% in the second, though there was varied response according to the respective concentration. As reported in the previous paper, a rough-dose response relationship was found. While sulphur dioxide was inhaled, the subjects complained of objective odor, irritation in the upper respiratory tracts, cough, and unusual sens(ation) in the lung. All the symptoms disappeared in a few minutes, and the airway resistance recovered to the first control value in 15-30 minutes."

"…the response of the inhalation of the mixture aerosols (H_2O_2 + SO_2) was more intense than that of the sulphur dioxide gas alone, especially in the first group exposed to the particles of larger size (4.6μ) the significant difference (p=0.01) was observed. The combination of sulphur dioxide and hydrogen peroxide aerosols was proved to respond synergistically to the respiratory tract."

"…it can be said that through the mouth, the larger size of the aerosol particles with CMD 4.6μ effects more intensively to the airway resistance than the smaller size of CMD 1.8μ."

*Statistically significant

Author's Conclusions:

Conclusions in each procedure enumerated in the abstracts are as follows:

1. **No changes of airway resistance were observed with hydrogen peroxide aerosol inhalation**
2. **airway resistance was increased as concentration of sulphur dioxide was increased**
3. **synergistic increase of airway resistance was induced by the mixture aerosol**
4. **airway resistance was decreased to below the control level**
5. **slight or no increase of the response was observed**

Review – Assessment

A. Subjects:	-✎ Is the number of subjects sufficient to produce statistically significant results?

	-✏ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ±✏ Are the subjects a representative sample of the population to which extrapolations will be made? ±✏ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? -✏ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	±✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✏ Was there a gradient of exposure levels? +✏ Were the chosen exposure levels appropriate to investigate the primary objective? -✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ±✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? -✏ Was evidence provided to justify the dosing regime? -✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	-✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✏ Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✏ Were the subjects randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✏ Was a control group assayed? ±✏ Is the control regime appropriate? +/-✏ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✏ Was the length of follow-up appropriate? +✏ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✏ Were appropriate endpoints examined? -✏ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) ±✏ Were accepted assessment methods employed? ±✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? +✏ Was raw data provided to permit the reader to arrive at his/her own conclusions?

	+✓ Was individual data reported for each subject? ±✓ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	±✓ Are the statistical methods used appropriate? -✓ Are confidence intervals reported? ±✓ Did the investigator appropriately interpret the statistics? +✓ Are the observed trends statistically significant?
G. Interpretations:	+✓ Was the original objective addressed? +/-✓ Are the results novel? ±✓ Was the design of the study appropriate to investigate the primary objective? -✓ Were assumptions reasonable? +✓ Are the methods reproducible? -✓ Did either the study design or the statistical analysis limit confounding variables? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings suggest that combined exposures of SO_2 and H_2O_2 aerosols have a synergistic effect in increasing airway resistance. Furthermore, larger sized aerosols (4.6μ) were shown to have a greater effect in increasing airway resistance than smaller (1.8μ) particles. However, airway resistance was the only respiratory parameter examined. The authors found a synergistic response between hydrogen peroxide aerosols and sulphur dioxide to pulmonary airway resistance. The resulting mixture of hydrogen peroxide and SO_2 presented as sulfuric acid aerosol. The text is confusing and it is unclear what the concentrations of the individual components are in the mix of the SO_2 gas and the H_2O_2 aerosol. However, a gradient was used in individual exposures of SO_2 from 1-60 ppm. The results of SO_2 alone show a dose response between SO_2 concentration and airway resistance. This study confirms findings from a Goetz study that demonstrated that sulfuric acid induced more intense response to pulmonary function than sulphur dioxide. However, due to small numbers of subjects in the study, results cannot be interpreted as statistically significant.

Confidence index:

This study can be seen to have lower confidence because, although the methods section describes the experimental apparatus well, it does not fully detail the characteristics of the study subjects, nor is there any mention of the statistical methods used to analyze the data. Also the exposures to the various aerosols occurred one-after-the-other on each individual, which may mean that effects may have carried over between experimental treatments. Furthermore, it is unclear of whether using a bronchodilator to measure airway resistance is appropriate. Also the analysis of respiratory parameters was restricted to only airway resistance and neglected other parameters. The SO_2 gradient was constructed by using either one or two subjects/ concentration point. This sample size is insufficient to provide statistically significant results. Also, no vigorous statistical treatment of the data was performed. All data were presented as absolute values. In general, a poorly designed experiment with invalid data analysis.

Review – Confidence index ranking:

Low

Author: Speizer, FE. and Frank, NR.	**Internal ID:** 054
Title: A Comparison of Changes in Pulmonary Flow Resistance in Healthy Volunteers Acutely Exposed to SO_2 by Mouth and by Nose	
Year: 1966	
Abstract: On separate occasions, healthy male volunteers were exposed either by nose or by mouth to one of two concentrations of sulphur dioxide, 15 and 28 ppm. Exposure to SO_2 lasted 10 minutes. Pulmonary flow resistance (RI) was measured by the oesophageal catheter method, and the lung volume was measured by a modification of the gas-compression method; when SO_2 was administered by nose, nasal flow resistance (Rn) was measured by means of a catheter placed in the posterior pharynx. The increase in RI was greater when SO_2 was administered by mouth than when it was administered by nose. Similarly, irritative symptoms of the posterior pharynx and chest were more common during exposure by mouth. These findings suggest that the mouth is less effective than the nose as an absorptive surface for SO_2.	
Objective: To compare changes in pulmonary flow resistance in healthy volunteers acutely exposed to SO_2 by mouth and by nose.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
15 to 28 ppm	10 minutes	male	unknown	8	healthy	Unknown	Unknown

Observations:

Pulmonary flow resistance (RI) increased significantly in 9 out of 12 experiments when SO_2 was administered by mouth. Often these changes were not sustained throughout the exposure. The magnitude of the change in RI was on average greater at 28ppm than at 15ppm During exposure to the same two levels of SO_2 by nose, RI increased significantly in only three out of 12 experiments and decreased in one experiment. In the period following administration SO_2 by mouth, RI remained significantly raised in five of the 12 experiments, four of which followed exposure to 28ppm. In 3 subjects, RI rose significantly for the first time during recovery. Approximately half the subjects breathing SO_2 by mouth had no measurable change in functional residual capacity (FRC). There was no consistent change in the nose patients. The response of nasal flow resistance was variable. "When exposed by mouth, most of the subjects coughed several times and had slight burning sensations of the throat and substernal area for at least five minutes. When exposed by nose, there was little coughing and no chest symptoms; some subjects did experience irritation of the posterior pharynx which lasted a few minutes.
*Statistically significant

Author's Conclusions:

"The administration of SO_2 by mouth to healthy subjects caused a greater rise in RI and more frequent coughing and discomfort of the posterior pharyngeal and substernal areas than when it was administered by nose. Findings suggest that the mouth may be less effective than the nose in removing SO_2 from the inspired air."

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+/-) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+/-) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+/-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+/-) Were the subjects randomly assigned to exposure groups? N/A Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+/-) Is the control regime appropriate? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and

	dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+/-) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+/-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+/-) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (+) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study indicated that the mouth was less effective than the nose as an absorptive surface for SO_2. Unfortunately, based on the small number of subjects involved, results cannot be considered statistically significant. Despite this, results were novel in that the differences in breathing gas by nose or mouth had not been previously investigated.

Confidence index:

This study does not make mention of many factors that may have confounded results, such as age or medical background. Furthermore, the number of subjects used (8) was not sufficient to produce statistically significant results. No mention is made of how subjects were recruited and whether they were informed of risks associated with the study. The exposure chamber is not described in the study, nor were environmental conditions indicated. There is no justification for the SO_2 concentrations used and there was a high variability between individual responses. Furthermore, data for the responses to control exposures was not included. Exposure duration was not clearly defined. Subjects had only been exposed for 1 minute before the first test was done. There is no justification for the

timing of assessment. The subjects were tested at 1, 5, and 10 min into exposure. The exposure duration seems too short. The length of recovery (or follow-up) was only 15 minutes. Subjects sat in a volume-displacement body plethysmograph during all the exposures by mouth and in only half of the exposures by nose; in the remainder, the subject sat outside the plethysmograph. There was no further discussion on why the subjects sat outside the chamber, or why this was done and how it was assigned.

Review – Confidence index ranking:

Moderate to low

DRAFT

Author: Trenga CA. Koenig JQ. Williams PV.	**Internal ID: 055**
Title: Sulphur dioxide sensitivity and plasma antioxidants in adult subjects with asthma.	
Year: 1999	

Abstract: OBJECTIVES: To screen adult subjects with asthma for sensitivity to inhaled sulphur dioxide (SO_2) and identify subject characteristics associated with that sensitivity. Medication use, symptoms, and plasma antioxidant nutrients between SO_2 responders and non-responders were compared. **METHODS:** Adult subjects (ages 18-39 years) with asthma were exposed to 0.5 ppm SO_2 for 10 minutes during moderate exercise. Pulmonary function tests and symptom ratings were assessed before and after exposure (n = 47). A subject was classified as sensitive to SO_2 if forced expiratory volume in 1 second (FEV1) showed a drop > or = 8% over baseline. Blood samples were obtained from subjects (n = 38) before the SO_2 challenge; plasma ascorbate, alpha-tocopherol, retinol, carotenoids, and lipids were measured. **RESULTS:** Of the 47 subjects screened, 53% had a drop in FEV1 > or = 8% (ranging from -8% to -44%). Among those 25 subjects, the mean drop in FEV1 was -17.2%. Baseline pulmonary function indices (FEV1% of predicted and FEV1/FVC% (forced vital capacity)) did not predict sensitivity to SO_2. Although use of medication was inversely related to changes in pulmonary function after SO_2 ($p < 0.05$), both SO2 responders and non-responders were represented in each medication category. Total symptom scores after exposure were significantly correlated with changes in FEV1 ($p < 0.05$), FVC ($p < 0.05$), and peak expiratory flow (PEF) ($p < 0.01$) but not forced expiratory flow between 25% and 75% vital capacity (FEF25-75). Plasma beta-carotene concentrations were inversely associated with PEF values and ascorbate concentrations were inversely associated with FEV1 and FEV1/FVC ($p = 0.05$ in all cases). High density lipoprotein concentrations were positively correlated with FEV1% of predicted ($p < 0.05$) and inversely correlated with change in FEF25-75 ($p < 0.05$) after SO_2. **CONCLUSION:** These results show that the response to SO_2 among adults with mild to moderate asthma is very diverse. Severity of asthma defined by medication category was not a predictor of sensitivity to SO_2. Lung function values were associated with beta-carotene and ascorbate concentrations in plasma; however, plasma antioxidant nutrient concentrations were not associated with sensitivity to inhaled SO_2.

Objective: To screen adult subjects with asthma for sensitivity to inhaled sulphur dioxide (SO_2) and identify subject characteristics associated with that sensitivity. Medication use, symptoms, and plasma antioxidant nutrients between SO_2 responders and non-responders were compared.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.5ppm	**10min**	**14M, 33F**	**18-39**	**47**	**Asthmatic**	**n/a**	**n/a**

Observations:

"The change in FEV1 after SO_2 challenge ranged from a small increase (6%) to a 44% decrement. Of the 47 subjects screened, 53% had a drop in FEV1 >= 8% (range -8% to -44%). The mean drop in FEF25-75 after the SO_2 challenge was -26% for the SO_2 responders, and -7% for the non-responders ($p<0.001$). Likewise the changes in FVC (-10% v -1%) and PEF (-15% v -2%) between the responders and non-responders were significant ($p=0.001$ and $p=0.002$, respectively). The SO_2 induced decrements in FEF25-75 and FEV1 were significantly greater ($p<0.05$) in subjects who used both bronchodilators and anti-inflammatory medication, compared with those who rarely used medication. Those who responded to SO_2 rated lower respiratory symptoms significantly higher than those who did not respond ($p<0.05$) after the SO_2 challenge: this was not the case for upper respiratory or other symptoms."

"Plasma beta-carotene concentrations were inversely associated with PEF values and ascorbate concentrations were inversely associated with FEV1 and

FEV1/FVC ($p = 0.05$ in all cases). High density lipoprotein concentrations were positively correlated with FEV1% of predicted ($p < 0.05$) and inversely correlated with change in FEF25-75 ($p < 0.05$) after SO_2."
*Statistically significant

Author's Conclusions:

"These results show that the response to SO_2 among adults with mild to moderate asthma is very diverse. Severity of asthma defined by medication category was not a predictor of sensitivity to SO_2. Lung function values were associated with beta-carotene and ascorbate concentrations in plasma; however, plasma antioxidant nutrient concentrations were not associated with sensitivity to inhaled SO_2."

Review – Assessment

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +✎ Are the subjects a representative sample of the population to which extrapolations will be made? +/-✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? ±✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	±✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
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	+✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
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G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The investigators collected blood samples before exposure of adult asthmatics to a low concentration of SO_2. The focus of the study was to identify factors such as medication use and nutrient levels and their effects on asthmatic response. This is a novel idea, yet many confounding variables, were not corrected for (see below). However, with this type of study, the control of many important variables may not be possible, thus limiting the clinical significance of this study. While these factors were not found to contribute to SO_2 sensitivity, the author recommends future research to determine possible associations between diet, plasma antioxidants and sensitivity to air pollutants.

Confidence index:

The study generally seems to be of good quality, with a good sample size (n=47) and included both men and women. While detailed methods were not presented here, references are provided to other published works. The inclusion of a control group (i.e. filtered air instead of SO_2) would have accounted for individual differences. The major flaw with this study was the lack of medical and/or lifestyle history to backup the blood plasma data. It is conceivable that persons with high levels of beneficial nutrients and low levels of LDL cholesterol may exercise more, drink less alcohol, have less stress, etc. In general, these results may reflect a lifestyle rather than a specific diet or usage of a specific drug. Also, asthma severity was classified by medication usage rather than by actual physician diagnosis, which may have led to some misclassification of subjects. No correction in the statistical analysis for these factors. No mention was made of the recruitment process, except to say that it targeted asthmatic subjects. Furthermore, it was not clear whether subjects were advised as to the risks associated with the study. Furthermore, conditions of the exposure system such as relative humidity and temperature were not indicated.

Review – Confidence index ranking:
moderate

Author: Wolff, RK., Dolovich, M., Rossman, CM. And Newhouse, MT.	**Internal ID:** 056
Title: Sulphur Dioxide and Tracheobronchial Clearance in Man	
Year: 1975	
Abstract: Tracheobronchial clearance was measured in nine healthy, nonsmoking adults. Technetium Tc 99m albumin aerosol was inhaled as a bolus under controlled conditions to achieve reproducible deposition in large airways. Each subject was studied in three separate three-hour experiments: twice under control conditions and once exposed to 5 ppm sulphur dioxide. Lung retention of activity was measured using a gamma camera interfaced to a data storage and retrieval system. The study indicated that (1) both deposition and clearance were highly reproducible in individuals in repeat control studies. (2) Acute exposure to 5 ppm sulphur dioxide had no significant effect on mucocilliary clearance in resting healthy subjects, except perhaps for a small transient change after one hour. Pulmonary function tests showed a decrease in maximal midexpiratory flow, but no other significant changes.	
Objective: To examine the acute effect of breathing 5 ppm sulphur dioxide on mucocilliary transport in the large airways of man by quantifying the removal of an inhaled radioactive aerosol.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
5 ppm	3 hours	Male and female	21-27	9	healthy	unknown	unknown

Observations:

Both deposition and clearance were highly reproducible in individuals in repeat control studies. Acute exposure to 5 ppm SO_2 had no significant effect ($P>.05$) on mucociliary clearance in resting healthy subjects, except perhaps for a small transient change ($P=.05$) after one hour. Pulmonary function tests showed a decrease in maximal midexpiratory flow ($P<.01$) but no other significant changes. No changes were found in vital capacity, forced expiratory volume at one second, or closing volume. Measurements of tracheal clearance were possible in some instances when sufficiently discrete centers of activity moved up the trachea. There was no apparent correlation between a cough in some subjects and increased clearance during sulphur dioxide exposure.

*Statistically significant

Author's Conclusions:

"We have studied the effect of acute exposure to 5 ppm sulphur dioxide alone, in resting healthy adults and have shown no marked effect on mucocilliary clearance. These results appear to confirm the suggestion by Amdur that sulphur dioxide in the pure gas form is a mild respiratory irritant. The real toxicologic danger from sulphur dioxide would appear to be its oxidation to sulphur trioxide and conversion to sulfuric acid in water droplets. Boren has suggested tha t carbon particles carry sulphur dioxide deep into the lung to produce enhanced effects. Indeed, the combination of sulphur dioxide, particulates, and water droplets has produced the most adverse effects. Further studies will be necessary to determine if these combinations affect mucocilliary transport."

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate

	for the objective of the study? (+/-) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+/-) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (-) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+/-) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Were the subjects randomly assigned to exposure groups? N/A Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+/-) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale?

	(+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results from this study indicated that 1) both deposition and clearance were highly reproducible in individuals in repeat control studies. (2) acute exposure to 5 ppm sulphur dioxide had no significant effect on mucocilliary clearance in resting healthy subjects, except perhaps for a small transient change after one hour and (3) pulmonary function tests showed a decrease in maximal midexpiratory flow, but no other significant changes. While this study was the first tracheobronchial study of its kind, its results must be interpreted with caution due to the low number of subjects in the study. . Further research would need to be conducted to confirm these findings. This study is for acute exposures only and in very healthy people breathing through their mouth. It would be difficult to extrapolate to the general population and inferences about chronic exposures cannot be made.

Confidence index:

This study does not make mention of how subjects were recruited to this study, or whether they were informed of risks prior to the study. No rational was given for the dosage of SO_2 used, and the sensitivity of the monitoring device was not recorded. While the study did make use of a control group, level of blinding was not indicated, nor was it clear if tests were randomly ordered. The sample size was small, however, the authors did include a power calculation indicating that the sample size was large enough to detect a 10% difference in retention which they deemed significant. The results of the experiment are critically dependent on reproducibility of clearance in a given individual since deposition site is probably the most important determinant of clearance rate. Reproducibility analysis showed that the technique was reproducible. The aerosol was inhaled as a bolus by a technique that achieves deposition in large airways only. It is not clear if there is a way to ensure that the entire aerosol is deposited in this area. In the exposure chamber, subjects' noses were stuffed with cotton batting and taped to prevent nose breathing. However, authors refer to studies indicating that mouth-breathing studies are relevant since most people breathe through their mouths

during exercise and some do at rest. It is unclear why the investigators chose radioactive tracing to monitor the end-point of clearance. Other techniques obviously have the sensitivity for this type of study. It is difficult to assess if this monitoring technique was adequate in measuring the desired end-point as there is no direct comparison.

Review – Confidence index ranking:

Moderate

Author: Sheppard D. Eschenbacher WL. Boushey HA. Bethel RA. **Internal ID: 057**

Title: Magnitude of the interaction between the bronchomotor effects of sulphur dioxide and those of dry (cold) air.

Year: 1984

Abstract: We studied the interaction between airway drying (cooling) and inhalation of sulphur dioxide (SO_2) causing bronchoconstriction in 8 subjects with mild asthma. On 3 separate days, we measured specific airway resistance (SRaw) before and after the subject performed voluntary eucapnic hyperpnea at a constant minute ventilation (30 to 40 L/min) for successive 3-min periods with doubling concentrations of SO2 in dry cold air (-20 degrees C, 0% relative humidity), in dry warm air (22 °C, 0% relative humidity), and in partially humidified warm air (22 °C, 70% relative humidity). On another day, we measured SRaw before and after the subject performed each of 6 successive 3-min periods of voluntary eucapnic hyperpnea at the same minute ventilation breathing dry cold air without SO_2. The concentration of SO_2 that caused a 100% increase in SRaw was significantly lower in dry cold air and in dry warm air than it was in humidified warm air. Repeated hyperpnea with dry cold air without SO_2 at the same ventilation had no effect on SRaw. We then had the same subjects perform voluntary eucapnic hyperpnea at successively increasing levels of ventilation on 3 different days with dry air alone, dry air with 0.1 ppm SO_2, or dry air with 0.25 ppm SO_2. The minute ventilation that caused an 80% increase in SRaw was significantly lower for hyperpnea with 0.1 and with 0.25 ppm SO_2 than for dry air without SO_2, but these differences were small. We conclude that sulphur dioxide causes bronchoconstriction at lower concentrations when it is inhaled in dry air than when it is inhaled in partially humidified warm air. Furthermore, at least with oral breathing, concentrations of SO_2 as low as 0.1ppm may cause modest potentiation of the bronchoconstriction produced by hypernea with dry air.

Objective: "We undertook this present study to determine the magnitude and significance of this interaction (that SO_2 exposure causes greater effects in cold dry rather than warm humid air) and to determine the relative contributions of decreased air temperature and decreased water content."

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.125-2.00ppm	**3min**	**5M, 3F**	**20-37**	**8**	**asthmatic nonsmoking**	**n/a**	**n/a**

Observations:

"Sulphur dioxide caused bronchoconstriction at a significantly lower concentration when it was inhaled by mouth during eucapnic hypernea in dry cold air or dry warm air than when it was inhaled in partially humidified warm air. The estimated mean PC_{100} was 0.51 ± 0.20ppm (mean ± SD) for SO_2 in dry cold air, 0.60 ± 0.41ppm for dry warm air, and 0.87 ± 0.41ppm for partially humidified warm air. The mean PC_{100} for dry cold air and that for dry warm air were not significantly different."

"When subjects inhaled 0.1 or 0.25ppm SO_2 in dry warm air, voluntary eucapnic hypernea caused bronchoconstriction at a significantly lower ventilation than it did when the subjects inhaled dry warm air without SO_2, though the differences in mean PV_{80} between these 3 conditions were small: PV_{80} was 54.9 ± 4.0L/min (mean ± SD) for air without SO_2, 51.1 ± 7.3 fir 0.1ppm SO_2, and 49.3 ± 6.7 for 0.25ppm SO2. As in the first part of the study, there was considerable intersubject variability."

*Statistically significant

Author's Conclusions:

"The results of this study show that SO_2 causes bronchoconstriction at a lower concentration when it is inhaled in dry air than when it is inhaled in humid warm air and that the potentiation of SO_2 induced bronchoconstriction by dry cold air is primarily a function low water content rather than low temperature. In addition, this study showed that a low concentration of SO_2 can potentiate the bronchoconstriction caused by hypernea with dry air because hyerpnea with dry air containing 0.1 or 0.25ppm SO_2 caused bronchoconstriction at a lower ventilation than did hypernea with dry air alone."

Review – Assessment

A. Subjects:	-✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +/-✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +/-✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the length of follow-up appropriate?

	+✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? ±✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +/-✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The authors studied the acute effects of temperature and humidity on the effect of SO_2 exposure at low concentrations. The results showed that dry cold conditions can cause bronchoconstriction at a lower SO_2 concentration. This finding is clinically significant and agrees well with other epidemiological studies which show increased responsiveness in the winter months to air pollution. However, given the small sample size, it is uncertain whether results could be considered statistically significant.

Confidence index:

The sample size for this study is small (n=8), but includes both men and women. The use of a gradient of SO_2 exposures to produce a dose-response relationship with bronchoconstriction was useful, and the use of ANOVA to analyze the results seems appropriate. The design of the study was well designed except for a few points. Each subject was exposed to increasing concentrations of SO_2 sequentially. Although impractical, it would have been prudent to perform a single exposure condition (temperature, humidity, concentration) / screening to ensure the previous exposures did not interfere with higher concentration exposures (induction effects). Also, the authors did not acclimatize the subjects before exposure to either dry or cold conditions. Although control measurements were made, the shock of the cold temperature or dryness may have been an aggravating factor that would enhance the toxicity of SO_2 even though such conditions may not induce bronchoconstriction. The subjects involved signed consent forms and were informed of the risks involved in the study. However, no mention is made of how they were recruited, or if any chose to withdraw from the study. And while randomization was incorporated into the study design, no mention is made of how the randomization was done.

Review – Confidence index ranking:

Moderate to high

Author: Sheppard, D., Nadel, JA. and Boushey, HA	**Internal ID:** 058

Title: Inhibition of Sulphur Dioxide-Induced Bronchoconstriction by Disodium Cromoglycate in Asthmatic Subjects

Year: 1981

Abstract: To determine whether disodium cromoglycate inhibits the bronchoconstriction produced by inhalation of sulphur dioxide in people with asthma, we undertook a study of 6 asthmatic subjects. Each subject inhaled 40 mg of cromolyn on one day and lactose placebo on another day 20 min before inhaling SO_2 for 10 min while exercising at a moderate rate on a bicycle ergometer. Sulphur dioxide was delivered in humidified air at ambient temperature in concentrations of 0.5 ppm (3 subjects) or 1.0 ppm (3 subjects). Cromolyn and lactose treatments were given to each subject in a randomized sequence and in a double-blind manner. On a third day, each subject exercised at the same work rate breathing humidified air without SO_2 at ambient temperature. We measured specific airway resistance in a body plethysmograph every 30 s for 10 min before and after each of the 3 periods of exercise. After treatment with lactose, SO_2 inhalation significantly increased SRaw in all 6 subjects. After treatment with cromolyn, SO_2 inhalation caused no increase in SRaw in 4 subjects and a small rise in 2 subjects. The mean increase in SRaw was significantly smaller than after lactose treatment. Exercise alone had no effect on SRaw in any subject. Thus, cromolyn inhibits SO_2-induced bronchoconstriction in subjects with asthma. These findings suggests either that SO_2 induces bronchoconstriction by stimulating the release of mediators from mast cells or that cromolyn inhibits bronchoconstriction by a mechanism independent of its effect on mast cells.

Objective: To study the effect of treatment with cromolyn on the bronchomotor response to SO_2 in subjects with asthma as a first step toward determining whether mast cell degranulation contributes to SO_2 induced bronchoconstriction.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.5 to 1.0 ppm	10 minutes/2 days one week apart	Male and female	22 to 30	6	Asthma	unknown	unknown

Observations:

After treatment with lactose, SO_2 inhalation significantly increased SRaw in all 6 subjects. After treatment with cromolyn, SO_2 inhalation caused no increase in SRaw in 4 subjects and a small rise in 2 subjects. The mean increase in SRaw was significantly smaller than after lactose treatment. Exercise alone had no effect on SRaw in any subject. Thus, cromolyn inhibits SO_2-induced bronchoconstriction in subjects with asthma.

*Statistically significant

Author's Conclusions:

"Our results suggested that cromolyn can inhibit reflex-mediated bronchoconstriction."

"Our results supported the view that SO_2 activates parasympathetic pathways indirectly by causing mast ells to degranulate."

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results?

	(+/-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive?

	(+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Findings from this study indicated that either SO_2 induces bronchoconstriction by stimulating release of mediators from mast cells or that cromolyn inhibits bronchoconstriction by a mechanism independent of its effect on mast cells. Results supported the view that SO_2 activates parasympathetic pathways indirectly by causing mast cells to degranulate. However, results cannot be considered statistically significant or applicable to other similar circumstances because of the small number of participants (six).

Confidence index:

The introduction of this study referenced only 4 studies, 2 of which were by the authors. This study successfully limited confounding factors prior to the study by recording subject statistics such as age and gender, and by requiring subjects to undergo screening tests of pulmonary function. Because method of recruitment is not mentioned, it is difficult to know how subjects came to know about the study. Furthermore, no mention is given for why six participants were used for the study, since more would have allowed results to be statistically significant.

Review – Confidence index ranking:

Moderate

Author: Stacy, R., Friedman, M., Hazucha, M., Green, J. | **Internal ID:** 060

Title: Effects of 0.75ppm Sulphur Dioxide on Pulmonary Function Parameters of Normal Human Subjects

Year: 1981

Abstract: Of 31 young, healthy male volunteers who participated in this study, 15 were exposed to air (control) and 16 to 0.75 ppm (2.15 mg/m^3) SO_2 for 2 hours at 21°C and 60% relative humidity. At the end of the first hour, the subjects exercised for 15 min on a treadmill at 6.4 kph, with a 10% grade. Methods employed in evaluation of pulmonary function included body plethysmography, spirometry, and multigas rebreathing test. From the battery of 15 pulmonary function parameters, only the pattern of airway resistance changes was significantly altered by SO_2 exposure, although spirometric parameters followed a similar pattern. Eight of the SO_2-exposed subjects, with one or more positive allergen skin tests, appeared to be significantly more reactive to SO_2 than skin test-negative subjects. All subjects remained asymptomatic. The small number of changes observed appeared to be reversible and do not suggest a significant health hazard to normal human subjects exposed to SO_2 under these conditions.

Objective: The objective of the study was to investigate the effect of SO_2 exposure on young, healthy subjects for 2 hours.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.75 ppm	2 hr	male	18-40	31	healthy	-	-

Observations:

In 14 of 16 SO_2-exposed subjects, RAW was increased between 2% and 55% at the end of the first hour of exposure, with a mean increase of approximately 14.6%. By contrast, the control group exhibited a 10.3% decrease in mean RAW. In general, other parameters exhibited a similar post-exercise pattern of response (decrease in the SO_2-exposed group and slight improvement in the air group.)

*Statistically significant

Author's Conclusions:

"It appears that SO_2 will elicit a measurable airways response in healthy subjects only when a certain rate of SO_2 loading is exceeded. Below this critical load, the respiratory system is apparently capable of neutralizing SO_2 and its metabolites rapidly enough that no immediate respiratory mechanics effects can be discerned."

"The small number of changes observed [after exposure to SO_2] appeared to be reversible and do not suggest a significant health hazard to normal human subjects exposed to SO_2 under these conditions."

"Although the data provided by our experiments give no information on the mechanisms of S)2 action, the elevated RAW and rend toward decreased FEF_{50} and $FEV_{1.0}/FVC$ implicate bronchoconstriction."

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+/-) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(+/-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+/-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and

	dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+/-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+/-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigates the effect of low dosages of SO_2 exposure on young, healthy subjects. Results indicate that only measures related to air flow resistance were significantly affected by SO_2 exposure. Upon exercise, decrements in lung function observed in the exposed group suggest SO_2-induced bronchoconstriction prevailed over exercise-induced bronchial relaxation. This research further reinforces the belief that exercise will have an adverse effect on pulmonary function in an SO_2-containing atmosphere. Information pertaining to the biological mechanism of SO_2 exposure was not given. Eight of the exposed study subjects that had one or more positive allergen skin tests appeared to be significantly more reactive to SO_2 (n=8), however this was not the objective of the study.

Confidence index:

The objective of the study was to determine the effects on a single SO_2 exposure (0.75 ppm) on healthy young male subjects. A calculation to determine an adequate sample size was not performed and therefore it is difficult to determine whether the statistical analysis lacks power. Subject screening consisted of comprehensive physical examinations, administration of a Minnesota Multiphasic Personality Inventory Test, and intradermal skin tests. The strict screening process may have resulted in sample bias. Specific information on why subjects were excluded should be included in the study. The study only looks at healthy male population and excludes sensitive sub populations. An analysis was done to determine if there was any difference in the effects of SO_2 exposure between subjects with positive allergen tests and negative tests, however, the sample sizes (n=8) were too small to determine significance. Confounders such as age, occupation etc. may be affecting the results. Occupational history for the subjects were not included. The study population was adequate to answer the study questions for that population, but cannot ensure representativeness of the general population and therefore may lack external validity. It is not apparent whether any blinding (single or double) occurred which will affect the internal and external validity of the study. The author provided raw data from the study, including age, height and weight and recorded reactions of subjects to intradermal injections of allergens. In addition to describing the exposure device, the author listed humidity, lighting and temperature conditions. The authors refer to other studies where similar results were shown. The study was designed to determine the short term effects of sulphur dioxide (at one concentration) in normal healthy men. The authors do not attempt to extrapolate to the general population and address the fact that the study does not give information regarding the biological mechanism. As far as showing that exposure to sulphur dioxide under these specific conditions, may cause bronchodilation, the study is adequate.The authors conclude that their results only imply bronchoconstriction and that furthur studies need to be done to determine the effects on sensitive subpopulations.

Review – Confidence index ranking:

High to moderate

Author: Sheppard D. Epstein J. Bethel RA. Nadel JA. Boushey HA | **Internal ID: 061**

Title: Tolerance to sulphur dioxide-induced bronchoconstriction in subjects with asthma.

Year: 1983

Abstract: A study to determine whether the bronchoconstriction induced by low concentration of sulphur dioxide in subjects with asthma decreases with repeated exposure was undertaken. Eight subjects with asthma performed 3 min of voluntary eucapnic hyperpnea with 0.5 ppm of SO_2 in humidified filtered air three times at 30-min intervals and we measured specific airway resistance (SRaw) before and after each period of hyperpnea. Specific airway resistance increased significantly more after the first exposure to SO_2 [(from 7.6 +/- 1.7 to 15.5 +/- 2.0 L x cm H2O/liter/sec (mean +/- SEM)] than after the second (from 8.1 +/- 1.3 to 10.8 +/- 1.6) or third (from 7.6 +/- 1.6 to 10.1 +/- 1.9) exposures (P less than 0.025). When seven subjects repeated hyperpnea with SO_2 24 hr and 7 days later, SRaw increased as much as it had after the first exposure (from 8.2 +/- 2.5 to 15.5 +/- 4.5 at 24 hr and from 6.6 +/- 1.4 to 15.4 +/- 2.1 at 7 days). In four subjects repeated exposure to SO2 caused short-term inhibition of the bronchomotor response to SO2 but did not inhibit the bronchomotor response to histamine aerosol. It was concluded that repeated exposures to a low concentration of SO_2 over a short period (on 1 day) can induce tolerance to the bronchomotor effects of SO2 in subjects with asthma. Tolerance to the bronchomotor effects of SO_2 is not caused by decreased responsiveness of airway smooth muscle or a generalized decrease in the responsiveness of vagal reflex pathways since the bronchomotor response to histamine is preserved.

Objective: "We therefore undertook this study to determine whether subjects with asthma develop tolerance to SO_2 induced bronchoconstriction and, if they do, how long this tolerance persists. In addition, we assessed the specificity of tolerance to SO_2 induced bronchoconstriction by studying whether or not decreased bronchomotor responsiveness to SO_2 is associated with decreased bronchomotor responsiveness to inhaled histamine."

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.5ppm	**3min / 30 min intervals**	**4M 4F**	**22-36**	**8**	**asthmatic nonsmoking**	**n/a**	**n/a**

Observations:

"When our subjects performed eucapnic hyperpnea with 0.5ppm of SO_2 three times in succession at 30-min intervals, the first exposure to SO_2 caused significantly more bronchoconstriction than did the second or third exposure. Twenty-four hours and 7 days later, the magnitude of SO_2 induced hyperpnea was the same as it had been initially. Voluntary eucapnic hyperpnea with partially humidified, ambient temperature air without SO_2 did not cause bronchoconstriction in our subjects."

"In four subjects, we assessed the effects of repeated exposures to SO_2 on the bronchomotor response to inhaled histamine aerosol. In each subject, the second period of hyperpnea with SO_2 caused less bronchoconstriction than the first. Despite this adaptation to the bronchomotor effects of SO_2, the bronchomotor response to inhaled histamine was not inhibited in any subject."

*Statistically significant

Author's Conclusions:

"This study shows that the bronchoconstriction caused by hyperpnea with 0.5ppm of SO_2 in subjects with asthma decreases with repeated exposures at 30min intervals: in other words, these subjects develop tolerance to the bronchomotor effects of SO_2. This decreased responsiveness (tolerance) to SO_2 persists for at

least 30min, but for less than 24hrs. Tolerance to inhaled SO_2 is not associated with decreased bronchomotor responsiveness to inhaled histamine."

Review – Assessment

A. Subjects:	-✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +/-✎ Are the subjects a representative sample of the population to which extrapolations will be made? +/-✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? +✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Were the subjects randomly assigned to exposure groups? +/-✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale?

	+✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study examined the induction of tolerance to SO_2 in asthmatic individuals. Repeated exposures of low levels (0.5 ppm) shows that brochoconstriction decreased in magnitude after repeated 30 min exposures. The tolerance is short lived and no protection could be found after one day. This tolerance was found not to protect histamine bronchoconstriction. This study is of clinical importance from two aspects. First, it shows that asthmatics who are not typically in an environment where there is low concentrations of SO_2 may be particularly sensitive to an acute exposure of SO_2. Second, the histamine test gives insight on the mechanism of SO_2 tolerance and possibly SO_2 bronchoconstriction.

Confidence index:

It is unclear in the paper how many subjects were used in the study. In the text, it refers to 10 subjects yet only 8 were shown in the table giving anthrompomorphic data. In either case, the number of subjects is small for the main study involving the tolerance study and even smaller (n=4) for the histamine challenge. The study controlled for confounders by selective screening and instructions for the subjects to refrain from intake of various medications. The design of the study was logical to examine the induction of tolerance yet it is unclear why only half of the subjects (n=4) were used for the histamine challenge. This portion of the study was important and a complete dataset would have made the data more convincing.

Review – Confidence index ranking:

moderate

Author: Tam, E.K., Liu, J., Bigby, B.G., Boushey, H.A. | **Internal ID:** 062

Title:Sulfur dioxide does not acutely increase nasal symptoms or nasal resistance in subjects with rhinitis or in subjects with bronchial responsiveness to sulphur dioxide

Year:1988

Abstract We examined whether brief exposures to moderately high concentrations of sulphur dioxide (SO_2) causes acute increases in nasal symptoms and nasal resistance in subjects with chronic rhinitis. We studied 19 subjects with allergic rhinitis and 3 subjects with chronic intermittent rhinorrhea, nasal congestion, and sneezing without any other manifestation of allergy. We found that the change in nasal resistance and symptoms caused by nasal inhalation of 4 ppm of SO_2 for 10 min was no greater than the changes caused by nasal inhalation of conditioned room air. In a second set of experiments, we examined whether allergic subjects with demonstrable bronchomotor responsiveness to SO_2 also had nasal responsiveness to the gas. We studied 8 subjects with a history of both asthma and allergic rhinitis. Each subject developed symptoms of dyspnea or wheezing and an increase in specific airway resistance of at least 8 L x cm H_2O/L/s after breathing 1 or 2 ppm of SO_2 by mouthpiece at 20 L/min, and did not develop these changes after breathing room air under the same conditions. No subject, however, developed more nasal symptoms or a greater increase in nasal airway resistance after tidally breathing SO_2 through the nose than after breathing room air, even when the concentration of SO_2 delivered to the nose was double the concentration delivered through the mouthpiece to the lower airways. We conclude that brief exposure to SO_2 at a concentration of 4 ppm or less is unlikely to cause significant nasal dysfunction in most subjects with chronic rhinitis, and that in subjects with both allergic rhinitis and asthma, responsiveness to SO_2 is not uniform throughout the respiratory tract.

Objective:

Study 1: To examine whether brief exposure to moderately high concentrations of SO_2 acutely provoke nasal symptoms and increases in nasal resistance n people with chronic or frequent sneezing, rhinorrhea, or nasal congestion induced by allergens or physical and chemical irritants.

Study 2: To examine whether bronchomotor responsiveness to the gas might be associated with increased nasal responsiveness. To do this we studied asthmatic subjects with proven bronchial responsiveness to SO_2. We reasoned that if the mechanisms responsible for such responsiveness were also present in the nose, these subjects would be likely to develop greater nasal symptoms or resistance changes after nasally breathing SO_2 than after breathing conditioned air.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Study 1: 4ppm	10 min	19 women 3 men	23-44 yr	22	Chronic Recurrent Rhinitis	N/A	N/A
Study 2: 2 ppm or less	4 minute	5 women 3 men	22-27 yr	8	Asthma	N/A	N/A

Observations:

Study 1:

The increase in nasal resistance caused by quiet breathing of 4 ppm SO_2 in filtered conditioned room air through a nasal mask was no greater than the increase in nasal resistance cause by quiet breathing of 4 ppm SO_2 in filtered conditioned room air through a nasal mask was no greater than the increase in nasal resistance caused by breathing filtered conditions room air alone.

Study 2:

Nasal symptoms and nasal resistance were no greater after breathing SO_2 than after breathing room air, irrespective of whether the gas was delivered through a mouthpiece or through a nasal mask.

Lower airway symptoms and SRaw were significantly greater after these subjects breathed SO_2 than after they breathed room air, confirming their bronchial responsiveness to SO_2. This SO_2 induced increase in SRaw was not associated with a change in nasal resistance.

*Statistically significant

Author's Conclusions:

" We conclude that brief exposure to SO_2 at a concentration of 4 ppm or less is unlikely to cause significant nasal dysfunction in most subjects with chronic rhinitis, and that in subjects with both allergic rhinitis and asthma, responsiveness to SO_2 is not uniform throughout the respiratory tract."

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-) Are the subjects a representative sample of the population to which extrapolations will be made? ((+/-) Was the method of recruitment appropriate? (No info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? (No info) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(+)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? (+)Were the chosen exposure levels appropriate to investigate the primary objective? (+/-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate?

	(+)Was the frequency of administration appropriate to investigate the primary objective? (+)Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+/-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Were the subjects randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+)Is the control regime appropriate? (+)Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (+)Was individual data reported for each subject? (+/-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible?

	(+/-)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study examined whether SO_2increases nasal symptoms or resistance in subjects with allergies (nasal) or bronchial responsiveness. The investigators did not find any evidence that SO_2 did not cause any nasal dysfunction with short exposures to SO_2 at up to 4 ppm. This study maybe clinically relevant from two points of view. First, the lack of response at the studied concentrations demonstrates that seasonal allergy sufferers are not at higher risk when exposed to typical pollution levels of SO_2. Second, the study shows that the mechanism of SO_2 toxicity is not associated with the nasal passages but further down in the bronchial tree. These findings are reasonable and reflect the data given in the paper. In Study 2, asthmatic subjects with documented bronchial responsiveness to SO_2 did not develop significantly greater increases in nasal symptoms or resistance after breathing SO_2 than after breathing room air. This study and studies mentionned in the discussion appear to the acute effects of SO_2 on nasal function are rarely clinically important at the concentrations used in this study.

This study may provide evidence as to the basic mechanisms of airway responsiveness. The results of this study differed somewhat from similar studies. Therefore, caution must be taken when comparing studies using different exposure levels, exposure duration, and exercise level, etc.

Confidence index:

The experimental design included a selective screening method for subjects before and during the study. This was clearly done to select for the most sensitive individuals, which increases the confidence in negative findings. Nevertheless, a control group should have been studied (non-atopic/asthmatic) so that the data could have been compared. The subject pool was not well distributed. The subjects were mostly female (19/22) and there was a wide range of IgE plasma concentrations. Also, the screening did not exclude important factors such as smoking, medication use etc. Although the investigators were aware of such variables, no effort was made to take this into account. Finally, it should be noted that the number of participants was not high enough to support statistically significant results.

Review – Confidence index ranking:

Moderate to High

Author: Andersen IB. Lundqvist GR. Jensen PL. Proctor DF | **Internal ID: 063**

Title: Human response to controlled levels of sulphur dioxide.

Year: 1974

Abstract: Nasal mucus flow rate, airway resistance, and subjective response was studied in 15 young men during 6 hour exposures to 1, 5, and 25ppm SO_2. A significant decrease in nasal mucus flow during 5 and 25ppm exposures was observed. This decrease was greatest in the anterior nose and in subjects with an initially slow mucus flow rate. Pharyngeal air samples yielded less than 1% of the SO_2 inhaled, even after a 6 hour exposure to 25ppm SO_2. An increased nasal airflow resistance and a fall in forced expiratory volume in one second and forced expiratory flow during the middle half of expired volume was found at all exposure levels, but there was no change in "closing volume." Discomfort was proportional to SO_2 concentration, but never excessive. Subjects with initially slow nasal mucus flow rates experienced the greatest discomfort. The acute effects of SO_2 exposure appear to justify reduction of the present threshold limit value of 5ppm to 1ppm, and provide support for a nasobronchial reflex bronchoconstriction.

Objective: "In this article, we describe a study designed to determine, in man, under carefully controlled conditions, whether the nose remains an effective filter for SO_2 over a period of hours, whether there are effects upon lung mechanics or the nasal airway or mucosa, and how much discomfort is associated with exposure to three concentrations of this material in clean air over six hours."

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1-25ppm	**6 hrs/day for 3 consecutive days**	**Male**	**20-28**	**15**	**healthy**	**n/a**	**1.0ppm**

Observations:

A significant decrease in nasal mucus flow rate during 5 and 25 ppm exposures was observed. This decrease was greatest in the anterior nose and in subjects with an initially slow mucus flow rate. Pharyngeal air samples yielded less than 1% of the So2 inhaled, even after a six-hour exposure to 25 ppm SO_2. AN increased nasal airflow resistance and a fall in forced exporatory volume in one second and forced expiratory flow during the middle half of expired volume was found at all exposure levels, but there was no change in "closing volume". Discomfort was proportional to SO_2 concentration, but never excessive. Subjects with initially slow nasal mucus flow rates experienced the greatest discomfort.

*Statistically significant

Author's Conclusions:

"The present study shows that 1ppm SO_2 during 1-6 hours does cause a constriction in the upper airways in young healthy subjects. An effect on the upper airways of chronic exposure to SO_2 concentrations below 1ppm therefore cannot be precluded."

Review – Assessment

A. Subjects:	+✐ Is the number of subjects sufficient to produce statistically significant results? +✐ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate

	for the objective of the study? ±✎ Are the subjects a representative sample of the population to which extrapolations will be made? +/-✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +/-✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	±✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? +✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Were the subjects randomly assigned to exposure groups? +/-✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? +/-✎ Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject?

	±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The investigators used 3 criteria to judge the human response to SO_2. The criteria complemented each other well, there was one quantitative end-point, one subjective end-point and one qualitative end-point. The results showed that there is a significant response of SO2 at 5 and 25 ppm shown by all 3 criteria. The use of radioactive tracing allowed tracking of mucus flow rates at different areas of the nasal passage. This finding is significant as it gave information on how SO_2 affected different parts of the nasal passage.

Confidence index:

The study was flawed in a number of aspects. The subject selection was not very stringent. The process did not exclude sensitive subjects such as smokers, atopics, asthmatics, etc. The exclusion criteria were somewhat vague and no medical history was noted. This would allow for confounders and no correction for this was taken in the statistical analysis. The exposure concentration was set a reasonable levels, however, the concentration of the SO_2 was gradually increased to the nominal test concentration. This would certainly allow the subjects to induce some sort of protective mechanism and the results may not reflect exposure to the test concentration. Also, since the SO_2 gradient was performed on 3 consecutive days, a tolerance may have been invoked. Results from day 2 and 3 may be influenced by previous exposures. Lastly the mode of exposure was not control for. All subjects were classified as nose breathers, yet this may have changed during the test period and could have been influenced by the possible irritation to the SO_2. It is apparent from previous papers that the route of SO_2 exposure strongly influences its effects and proper control of this factor is necessary to provide accurate results. This study makes no mention of how subjects were recruited or if they were warned of experimental risks. However, it does explain that randomization of exposures was not done in order to limit adverse health effects from subjects.

Review – Confidence index ranking:

Moderate

Author: Balmes, JR., Fine, JM. And Sheppard, D.	**Internal ID:** 064

Title: Symptomatic Bronchoconstriction after Short-Term Inhalation of Sulphur Dioxide

Year: 1987

Abstract: We studied the relationship between duration and concentration of exposure in SO_2-induced bronchoconstriction in 8 asthmatic subjects. On separate days, we administered SO_2 in humidified air through a mouthpiece at 2 concentrations (0.5 and 1.0 ppm) for 3 time periods (1, 3, and 5 minutes) during eucapnic hyperpnea (60L/min). Humidified air was administered for 5 min as a control. Bronchoconstriction was assessed by measurement of specific airway resistance. The magnitude of the bronchoconstrictor response to both concentrations of SO_2 increased progressively over the 3 time periods studied. The mean increase in SRaw (in L x cm H_20/L/s) and percent increase above baseline after each exposure to SO_2 were as follows: 2.5 +/- 0.3 (34%) after 0.5 ppm for 1 minute; 7.5 +/- 4.7 (93%) after 1.0 ppm for 1 min; 13 +/- 3.2 (173%) after 0.5 ppm for 3 min; 31.4 +/- 7.4 (395%) after 1.0 ppm for 3 min; 19.6 +/- 4.0 (234%) after 0.5 ppm for 5 min; 44.1 +/- 9.8 (580%) after 1.0 ppm for 5 min; 3.5 +/- 1.5 (46%) after humidified air for 5 min. For the group, the increases in SRaw caused by inhalation of both concentrations of SO_2 for 1 min were small. However, 2 of 8 subjects did develop large increases in SRaw and chest tightness after inhalation of 1.0 ppm for 1 min. Seven of 8 subjects developed wheezing, chest tightness, or dyspnea and used an inhaled bronchodilator after inhalation of 0.5 ppm for 3 and 5 min and 1.0 ppm for 3 minutes. Two subjects were unable to complete the 5-min exposure to 1.0 ppm because of symptomatic bronchoconstriction. These results suggest that short-term inhalation of near ambient concentrations of SO_2 can cause clinically significant bronchoconstriction. If these effects are confirmed in freely breathing subjects, pollution control strategies may need to be devised to prevent such short-term peak concentrations of SO_2 from occurring in ambient air.

Objective: To study the relationship between duration and concentration of exposure in SO_2-induced bronchoconstriction.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.5 and 1.0 ppm	1,3 and 5 minutes	Male and female	23 to 39	8	asthma	unknown	0.5 ppm

Observations:

Inhalation of both concentrations of SO_2 for all 3-time periods studied caused significant increases in SRaw above baseline. However, the effects of 1-min exposures were primarily confined to 2 subjects whom developed chest tightness and a marked increase in SRaw after a 1-min exposure to 1ppm SO_2. After each 3 and 5 min exposure, 7 of 8 subjects developed large increases in SRaw. Over the time periods and concentrations employed in this study, the increases in SRaw caused by SO_2 were both time and concentration dependent. Thus, for both concentrations, SRaw increased significantly more after 3 min than after 1 min and also increased significantly more after 5 min. For the 3 and 5-min time periods, the effect of 1ppm was significantly greater than that of 0.5ppm. Most subjects developed small increases in SRaw after 5 min of hyperpnea with humidified, filtered air that were significantly less than the increases caused by inhalation of both concentrations of SO_2 for 5 min ($p<.025$).

*Statistically significant

Author's Conclusions:

" These results suggest that short-term inhalation of near ambient concentrations of SO_2 can cause clinically significant bronchoconstriction. If these effects are confirmed in freely breathing subjects, pollution control strategies may need to be devised to prevent such short-term peak concentrations of SO_2 from occurring in ambient air."

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (+/-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random Assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate?

	(+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in this document?

Review - Summary:

Discussion of findings:

The results of this study are convincing yet they do not provide additional information about SO_2 induced bronchoconstriction. The authors point out that brnchoconstriction has been noted in asthmatic subjects at short exposures of relatively low concentrations. The study did not observe co-contributing factors such as ventilation rate or exposure route and thus does not contribute to any new clinical findings. The conclusions are ambitious as they state that results of this nature may influence legislation on SO_2 emissions in the environment. The main issue against this is the route of exposure used in the study. Direct mouth breathing for SO_2 exposure greatly enhances bronchoconstriction and may not be a valid measure of the impact to human health.To achieve statistically significant results, similar future studies should use more than 8 subjects. Most previous studies have examined duration of exposure of 5 min or greater. It appears, from the results in this study, that the magnitude of SO_2 induced bronchoconstriction was clearly shown to increase directly with the duration of exposure for periods of 5 min or less, for both concentrations studied. The early time of SO_2 induced bronchoconstriction may have important regulatory implications, if 5 – 10- min peaks exceeding certain concentrations occur. However, the results need to be confirmed with unencumbered breathing.

Confidence index:

This study was well designed, apart from the low number of subjects. Only 6 of the 8 subjects were able to participate in the 1 ppm exposure for 5 min. A gradient of doses was administered and a control group assayed. While these tests were randomly administered, no mention was made of the randomization method. However, Many confounders were accounted for and there was stringent control of experimental variables.

Review – Confidence index ranking:

Moderate

Author: Coe JE, Douglas RB	**Internal ID: 065**
Title: The effect of contact lenses on ocular responses to sulphur dioxide.	
Year: 1982	
Abstract: n/a	
Objective: "It is the purpose of the present investigation to attempt to provide a rational basis for developing guidelines on the wearing of contact lenses at work by determining the effect of impermeable ('hard') corneal contact lenses on the tear-producing response of the human eye to the common industrial irritant gas, sulphur dioxide."	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
50ppm	**5min**	**3M 3F**	**22-40**	**6**	**healthy**	**n/a**	**50ppm**

Observations:

"In all subjects, exposure of the unshielded eye (without contact lens) to 50parts/10^6 sulphur dioxide led to an increase in the level of tear production as measured in the shielded eye, with a mean increase of 83%. This increase in tear production was significant ($p<0.01$). Exposure of the shielded eye (with contact lens) to 50parts/10^6 sulphur dioxide produced no significant change in the level of tear production in any subject."
"…no subjects reported any subjective sensation of eye irritation with exposure of the shielded or unshielded eye."

*Statistically significant

Author's Conclusions:

"…If as is mandated for industries with eye hazards, protective spectacles are worn in work areas, the necessity for stringent restrictions against the wearing of contact lenses at work would seem to be less pressing."

Review – Assessment

A. Subjects:	-✐ Is the number of subjects sufficient to produce statistically significant results? +/-✐ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +/-✐ Are the subjects a representative sample of the population to which extrapolations will be made? ±✐ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? ±✐ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	±✐ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✐ Was there a gradient of exposure levels? +✐ Were the chosen exposure levels appropriate to investigate the primary objective?

	-✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? -✏ Was evidence provided to justify the dosing regime? ±✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined - Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✏ Were the subjects randomly assigned to exposure groups? +/-as the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? -✏ Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) +✏ Was the length of follow-up appropriate? +✏ Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	+✏ Were appropriate endpoints examined? ±✏ Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? +✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✏ Was individual data reported for each subject? ±✏ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	±✏ Are the statistical methods used appropriate? -✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
G. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? -✏ Was the design of the study appropriate to investigate the primary objective?

	±✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? + Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

By using tear production as an endpoint to measure the irriative nature of SO_2, the investigators found a protective effect of impermeable contact lenses. The findings in this study are conclusive but are not clinically relevant. Although it is clear from the data that hard contact lenses provide a physical barrier towards SO_2 gas, it does not study the synergistic effects of SO_2 and other aerosols that could be present in the workplace. Such interaction may proceed to make other chemicals (like sulphuric acid), which could concentrate in the lens and thus cause damage to the eye. Persons were only exposed for 5minutes, and as such, the study may have neglected to see effects that may have resulted from longer-term exposures. The conclusions made by the authors are not warranted given the simplicity of the study

Confidence index:

The sample size of this study is small (n=6) and the exposures were of very short duration (5min), making it very difficult to draw strong conclusions from its findings. The experiments were to designed to measure only one variable: tear production. It appears that this measurement was adequately performed with the procedure given in the text. It is unclear what the true concentration of the SO_2 was as it was not measured inside the exposure device. The endpoint had few confounders and subjects were chosen to limit the possibility . Statistical analysis was performed but the method of analysis was not stated. The exposure concentration is relatively high and it is not stated why such an exposure concentration was used. While confounding factors such as age and health were accounted for, investigators did not record the occupational profiles of the volunteers that might have impacted results. Furthermore, no rationale was given for the exposure level used in this study, and descriptions of monitoring devices and sensitivity was not given.

Review – Confidence index ranking:

Low

Author: Bechtold, WE., Waide, JJ., Sandstrom, T., Stjernberg, N., McBride, D., Koenig, J., Chang, I. And Henderson, RF.	**Internal ID:** 066

Title: Biological Markers of Exposure to SO_2: S-Sulfonates in Nasal Lavage

Year: 1993

Abstract: S-sulfonate levels were measured in the nasal lavage (NAL) fluid of humans exposed to sulphur dioxide as a potential biological marker of exposure. These levels were determined by treating NAL fluid protein with cyanide to cleave the S-S linkage and release the sulfite. The cyanolytically released sulfite was measured by ion chromatography. In two experiments, humans were exposed to air or 1 ppm SO_2 for 10 minute, and to air or 7 ppm SO_2 for 20 minutes and lavaged immediately after exposre. Releasable sulfite levels in NAL fluid were 1.06 +/- 0.24 and 2.61+/- 0.55 µg SO_2/mg protein, respectively, for the first experiment, and 1.16 +/- 0.37 and 4.91 +/- 0.76 µg SO_3/mg protein, respectively for the second. The subjects in the former study were persons with asthma. In both experiments, S-sulfonate levels were statistically elevated in the exposed group compared with the control groups. The same individuals in the second experiment received five additional 20-minute exposures to 7 ppm SO_2 every other day, for a total of six exposures. NAL fluid taken at the conclusion of the final exposure had releasable sulfite levels of 4.99 +/- 1.36 µg/SO_3/mg protein; these levels were statistically elevated relative to controls but were not elevated relative to the 1-day exposure. The lack of accumulation of S-sulfonates after 6 days of short-term exposure suggests clearance of these compounds from the nasal passage within 24 hours. The levels of S-sulfonates observed in NAL fluid in this study are almost three orders of magnitude higher than those measured in plasma following similar SO_2 exposures. Measurement of S-sulfonates in the nasal passage may be an effective short-term biomarker of exposure to SO_2.

Objective: To measure S-sulfonate levels in the nasal lavage (NAL) fluid of humans exposed to sulphur dioxide as a potential biological marker of exposure.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1 or 7 ppm	10 to 20 minutes/every other day/3 weeks	Male and female	13 to 35	5/8	asthma	unknown	unknown

Observations:

In both experiments, S-sulfonate levels were statistically elevated in the exposed group compared with the control groups. The same individuals in the second experiment received five additional 20-minute exposures to 7 ppm SO_2 every other day, for a total of six exposures. NAL fluid taken at the conclusion of the final exposure had releasable sulfite levels of 4.99 +/- 1.36 µg/SO_3/mg protein; these levels were statistically elevated relative to controls but were not elevated relative to the 1-day exposure. The lack of accumulation of S-sulfonates after 6 days of short-term exposure suggests clearance of these compounds from the nasal passage within 24 hours. The levels of S-sulfonates observed in NAL fluid in this study are almost three orders of magnitude higher than those measured in plasma following similar SO_2 exposures.

*Statistically significant

Author's Conclusions:

"Our results show that measurement of S-sulfonates in NAL can be effective as a biological marker of recent exposure to SO_2."

"These results demonstrate that S-sulfonates can only be used as a biological marker for expsoures that have occurred within 24 hours."

"These results suggest that assay of cyanolyitcally releasable sulfite levels in NAL fluid can be an effective means for biomonitoring for SO_2 exposure."

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+/-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+) Was a full description of the monitoring devices provided? (+) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Were the subjects randomly assigned to exposure groups? N/A Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random Assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc)

	(+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+/-) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+/-) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? N/A Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in this document?

Review - Summary:

Discussion of findings:

The study investigated the use of releasable S-sulfonates as a biomarker for SO_2 exposure. Clinically this is not important as many conceivable chemicals can react with disulphides on proteins (the "sink" for capature of SO_2). Also, the concept of "releasable" was not defined properly in the text and there was not a effort to find S-sulfonates in the sample what still remained in the nose. Results from this study indicate that assay of cyanolytically reasonable sulfite levels in NAL fluid can be an effective means for monitoring SO_2 exposure. While the results suggest that the assay can be applied to occupational and environmental exposures as low as 1 ppm, this study used only 13 subjects, insufficient to support statistically significant results. Because the human exposures took place in two different laboratories with different techniques and under different conditions, it is impossible to compare the reslts between the two grups. From these results, it does not appear that S-sulfonate is not appropriate for exposures that take place longer than 24 hours.

Confidence index:

This investigation recorded confidence factors such as participant age, gender, and overall health. However, no mention was made of the recruitment process. Furthermore, despite the limited number of participants, individual data was not reported for each subject. The experimental design was not adequate for proving that s-sulphonates in nasal lavage could be used as a biomarker. For this type of experiment, strict control of confounders is necessary and this was not the case in the study. Subjects needed to be isolated before the collection of a control sample as any exposure to environments with sulphur dioxide (or sulfurous chemicals in general) such as smoky areas, exposure to smokers, work environments etc. To fully prove the utility as a biomarker, a dose-response curve must be constructed. In this study, only two data points in the two studies were collected: the control and test point. The test itself is invalid as it measures the SO_2 concentration /mg of protein. It is reasonable to assume that the protein concentration is much higher than the SO_2 concentration encountered and persons with exceptional protein secretion would skew this result. Also, individual differences would cause for differences in the types of protein secreted in the nasal passage. Each type of protein has different numbers of disulphide bonds; the functionality in which the SO_2 binds onto. Inter and intra individual variability in the exposed and unexposed was not discussed.

Review – Confidence index ranking:

Moderate

Author: Devalia JL. Rusznak C. Herdman MJ. Trigg CJ. Tarraf H. Davies RJ.	**Internal ID: 067**
Title: Effect of nitrogen dioxide and sulphur dioxide on airway response of mild asthmatic patients to allergen inhalation.	
Year: 1994	
Abstract: Air pollution may enhance the airway response of asthmatic subjects to allergen inhalation. To test the hypothesis that sulphur dioxide and nitrogen dioxide alone or in combination could have a contributory role, we have studied the effect of 6 h exposure to air, 200 parts per billion (ppb) sulphur dioxide, 400 ppb nitrogen dioxide, and the two gases together on the airway response to inhaled allergen in ten volunteers with mild atopic asthma. The subjects were exposed to the gases in random order at weekly visits, then challenged with pre-determined concentrations of Dermatophagoides pteronyssinus allergen 10 min after each exposure. The forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), and cumulative breath units (CBU) of D pteronyssinus allergen required to produce a 20% fall in FEV1 (PD20FEV1) were measured after each exposure. Compared with air, neither sulphur dioxide nor nitrogen dioxide nor the combination significantly altered FEV1 or FVC. Although the decreases in PD20FEV1 after exposure to each agent alone were not significant (41.2%, p = 0.125 after nitrogen dioxide; 32.2%, p = 0.506 after sulphur dioxide) the decrease after exposure to the combination was significant (60.5 [SE 8.1]%, p = 0.015). Exposure to a combination of sulphur dioxide and nitrogen dioxide in concentrations that could be encountered in heavy traffic enhances the airway response to inhaled allergen, possibly as a result of previous airway inflammation.	
Objective: "We have studied the effect of low concentrations of gases on the airway response of people with mild asthma to inhaled house-dust-mite (HDM) antigen."	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
200ppb	**6 hours**	**4M 4F**	**18-45**	**10 started** **8 finished**	**Mild asthma**	**200ppb**	**n/a**

Observations:

"…in comparison with measurements after exposure to air, the only significant reduction (in $PD_{20}FEV_1$) was for the combination of nitrogen dioxide and sulphur dioxide (% change 60.5 (SE 8.1%), p=0.015). However, mean $PD_{20}FEV_1$ was reduced after exposure to nitrogen dioxide (41.2%, p=0.125) or sulphur dioxide (32.2%, p=0.506) singly."
*Statistically significant

Author's Conclusions:

Exposure to a combination of sulphur dioxide and nitrogen dioxide in concentrations that could be encountered in heavy traffic enhances the airway response to inhaled allergen, possibly as a result of previous airway inflammation.

Review – Assessment

A. Subjects:	-✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate

	for the objective of the study? +/-✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? +✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+/-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? +✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✎ Was individual data reported for each subject?

	+✏ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✏ Are the statistical methods used appropriate?
	-✏ Are confidence intervals reported?
	+✏ Did the investigator appropriately interpret the statistics?
	±✏ Are the observed trends statistically significant?
G. Interpretations:	+✏ Was the original objective addressed?
	-✏ Are the results novel?
	+✏ Was the design of the study appropriate to investigate the primary objective?
	+✏ Were assumptions reasonable?
	+✏ Are the methods reproducible?
	+✏ Did either the study design or the statistical analysis limit confounding variables?
	+✏ Do the conclusions make biological sense?
	-✏ Are the extrapolations reasonable?
	+✏ Is the paper in a peer-reviewed journal?
	+✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the effect nitrogen and sulphur dioxide on airway response alone or in combination on subjects challenged to allergen. The results show that only the combination of the two gases gave a statistically significant response and not alone. These results are not clinically significant, as the study did not examine the possible synergistic effect of the two gases. The result could have been due to an additive effect; i.e. the irritant level was increased above a certain threshold value.

Confidence index:

The most important concept in this paper was difficult to understand. The concept of the cumulative breath unit was used to construct dose-response curves so that different experimental conditions could be compared. The construction of dose-response curve consisted of 2 points on a log scale. This type of extrapolation is not valid and required additional data to provide a more accurate curve. The experimental design as reported was flawed in a number of ways. The number of subjects was small (n=8) and the result with the combined gases was barely significant. More test subjects may have made the data more convincing. The exposure conditions are questionable, as the chamber was not described and the humidity and temperature of the exposure not given. No information was given about how the subjects breathed in chamber. This would have a dramatic effect on the response to the gases.

Review – Confidence index ranking:

Low to moderate

Author: Snell, R.E., Luchsinger, P.C.	**Internal ID:** 070
Title: Effects of sulphur dioxide on expiratory flow rates and total respiratory resistance in normal human subjects	
Year: 1969	
Abstract None	
Objective: The present study was designed to observe the acute effects of SO_2 in the 0.5-5.0 ppm range, both alone and in combination with saline or water aerosols.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
First Series: 1. Aerosols of distilled water and normal saline 2. SO_2 in air at 0.5,1,5 ppm 3. SO_2 at 0.5,1,5 mixed with a normal saline aerosol 4. SO_2 at 0.5,1,5 ppm mixed with a distilled water aerosol	15 minutes each (15 minute control period preceded the challenges)	5 male 4 female	20-40 yrs	9	Healthy (3 smokers)		
Second Series: 1. 5 ppm SO_2 in air 2. 0.5ppm SO_2 in distilled water aerosol **3.** 5 ppm SO_2 in a distilled water aerosol	15 minutes	No info	No info	5 (3 of which were in first series)	No info		

Observations:

First Series:

The normal saline aerosol produced no significant change in the maximum expiratory flow from one half vital capacity ($MEF_{50percentVC}$) when compared to the control values.

With the distilled water aerosol, the group showed a small but significant increase in flow rates following exposure.

The decrease in $MEF_{50percentVC}$ after inhaling SO_2 mixtures in air was significant at the 1 and 5 ppm levels.

When mixed with normal saline aerosol, the fall in $MEF_{50percentVC}$ was significant only at the 5-ppm level. The response to the gas alone and to the gas mixed with the saline aerosol were qualitatively similar. With the distilled water aerosol, the fall in flow rates in comparison to the control was significant at all three levels of SO_2.

Second Series:

The average $MEF_{50percentVC}$ was higher when exposure was through a mask rather than a mouthpiece. This was highest when only SO_2 gas was inhaled.

During mouth inhalation of SO_2, a fall in conductance (G_{rs}) of approximately 25% was observed both for the gas alone and in combination with the water aerosol. A similar drop was noticed when SO_2 was administered by mask.

Nasal inhalation of the dry gas did produce an increase in total respiratory resistance, the drop in $MEF_{50percentVC}$ that occurred following exposure by mouth to the same gas mixture, was largely absent.

Exposure to the gas-aerosol combination at the same SO_2 concentration resulted in an increase in total respiratory conductance and a fall in flow rates only slightly less than that seen following mouth breathing.

*Statistically significant

Author's Conclusions:

"The comparison between nasal and mouth exposure to the SO_2 – water aerosol combination produced effects that, to our knowledge, have not been observed previously in animals or man. While nasal inhalation of the dry gas produced the changes described above, exposure to the gas-aerosol combination at the same concentration resulted in an increase in total respiratory conductance and a fall in flow rates only slightly less than that seen following mouth breathing."

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+/-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study?

	(-)Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? (No info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (-) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? (+)Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (-)Was the frequency of administration appropriate to investigate the primary objective? (+/-)Was evidence provided to justify the dosing regime? (-)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-)Was a full description of the monitoring devices provided? (-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Were the subjects randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+)Is the control regime appropriate? (+)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+/-)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+/-)Were assessment measures sufficiently sensitive? (+/-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-)Did qualified personnel conduct the exams?

	(+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (+/-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+/-)Are the statistical methods used appropriate? (-)Are confidence intervals reported? (+/-)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (+/-)Are the results novel? (+/-)Was the design of the study appropriate to investigate the primary objective? (+/-)Were assumptions reasonable? (+/-)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables? (+/-)Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The decrease in $MEF_{50percentVC}$ after inhaling SO_2 mixtures in air was significant at the 1 and 5 ppm levels. When mixed with normal saline aerosol, the fall in $MEF_{50percentVC}$ was significant only at the 5-ppm level. The responses to the gas alone and to the gas mixed with the saline aerosol were qualitatively similar. With the distilled water aerosol, the fall in flow rates in comparison to the control was significant at all three levels of SO_2. It appears that the ability of the aerosol to potentiate the effect of SO_2 may depend on the particle size, which would depend on the method used to create the aerosol.. In the second series of the study, the differences between mouth and nose inhalation were tested. There were no significant findings in this series. This study was possibly the first to indicate the synergistic effect of aerosols in conjunction with SO_2 and the significance of the route of inhalation. It is not apparent that the investigators designed the study to investigate both factors together, however, they are definitely related.

Confidence index:

A number of aspects of the experimental procedure were not evident in the text. The exposure equipment was not detailed along with the temperature and the amount of particulates in the exposure gas. These factors would be very important to study the objective completely and were probably omitted because the authors were unaware of their importance. The number of subjects was small (n=9) and little medical history or individual data was given except that they were non-asthmatic or allergic. 3 were smokers: this represents a large proportion of the subjects. These factors may have lead to confounding variables that were not controlled for. Lastly, there was no mention in the text about the statistical techniques used to give the levels of significance. All of the subjects were either physicians or technicians with some experience in pulmonary function testing.

In the first series, the subjects were volunteers. In the second series, 3 of the five subjects were from the first series, and there is no information on the other 2. There is no information on any of the 5 subjects in the second series. It is not clear if there was a control period in the second series. The time between challenges was not indicated. Responses between the SO_2 inhalation and the SO_2 – distilled water aerosol were only qualitatively similar. No test for significance.
The second series of tests involving the difference between nose exposure and mouth exposure was not included in the introduction or study objective.

Review – Confidence index ranking:

Moderate to Low

DRAFT

Author: Kagawa J.	**Internal ID:072**

Title: Respiratory effects of two-hour exposure with intermittent exercise to ozone, sulphur dioxide and nitrogen dioxide alone and in combination in normal subjects.

Kagawa J.

Year:1983

Abstract: Seven adult male healthy volunteer subjects were exposed to 0.15 ppm each of O_3, SO_2 and NO_2 alone and in combination, with intermittent light exercise for two hours. Three of the 7 subjects developed cough during deep inspiration and one subject had chest pain during exposure to O_3 alone. Among the various indices of pulmonary function tests, specific airway conductance (Gaw/Vtg) was the most sensitive index to examine the changes produced by the exposure to O_3 and other pollutants. Significant decrease of Gaw/Vtg in comparison with control measurements was observed in 6 of 7 subjects during exposure to O_3 alone, and in all subjects during exposures to the mixture of O_3 and other pollutants. However, no significant enhancement of effect was observed in the mixture of O_3 and other pollutants, although a slightly greater decrease of Gaw/Vtg was observed for the mixture of O_3 and other pollutants than for O_3 alone.

Objective: This paper investigates the possible interactions among mixtures of ozone and other pollutants at a lower concentration (0.15) than 0.37 ppm

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.15 ppm	2 hours	male	19-23	7	Healthy	NA	NA

Observations:

There were no symptoms associated with exposure to SO_2 or NO_2.

Significantly decreased specific airway conductance (G_{aw}/V_{tg}) was observed in 4 subjects during SO_2 exposure and 2 subjects during SO_2+NO_2 exposure, and all subjects during O_3+SO_2 and $O_3+SO_2+NO_2$ exposure.

One-hour post exposure, a significant decrease of G_{aw}/V_{tg} was observed in 2 subjects for SO_2+NO_2 exposure, one subject for SO_2 exposure, 3 subjects for O_3+NO_2 exposure and 3 subjects for $O_3+SO_2+NO_2$.

A significantly enhanced effect ($P<.01$) was observed in 3 subjects during O_3+SO_2 exposure and in one subject during $O_3+SO_2+NO_2$ exposure in comparison with O_3 exposure (at one hour into exposure).

One hour post-exposure, a significantly enhanced effect ($p<.01$) was observed in subject 3 after O_3+SO_2 exposure, and in subject 3 after $O_3+SO_2+NO_2$ exposure in comparison with O_3 exposure. The significantly enhanced effect ($p<.01$) during $O_3+SO_2+NO_2$ exposure in comparison with O_3+SO_2 or O_3+NO_2 exposure was observed in 2 subjects.

The mean values of pulmonary function tests at one hour exposure (E1), two-hour exposure(E2) and one hour postexposure (R1) were compared with control values.

As for SO_2 exposure, significant increases were observed in functional residual capacity (FRC) and residual volume (RV) of R1. As for SO_2+NO_2 exposure, significant decreases were observed in G_{aw}/V_{tg} of E2, $FEV_{1.0\%}$ of E1 and E2.

The test that produced the greatest number of statistically significant effects was G_{aw}/V_{tg}. Although several indices of pulmonary function tests other than G_{aw}/V_{tg} showed significant changes during the exposure to pollutant gases, their changes were small.

Although a slightly greater decrease of G_{aw}/V_{tg} was observed during the exposure to the mixture of O_3, SO_2, and NO_2 in comparison with O_3 exposure, no significant difference was observed among O_3, O_3+SO_2, O_3+NO_2, and O_3+SO_2+NO_2.

*Statistically significant

Author's Conclusions:

"The present study suggests a slightly more severe effect of O_3+SO_2 than with O_3 alone in a group of volunteer subjects exposed to a lower concentration of 0.15ppmO_3+0.15ppmSO_2 than 0.37 ppm O_3 and 0.37ppm SO_2."

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results?(-) (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study?(-) (-) Are the subjects a representative sample of the population to which extrapolations will be made?(-) (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?(NA) (+/-) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?(NA)
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+/-) Was evidence provided to justify the dosing regime?

	(+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined? (+) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Were the subjects randomly assigned to exposure groups? (+/-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+/-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+/-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+/-) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+/-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+/-) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (+/-) Were assumptions reasonable? (+/-) Are the methods reproducible?

	(-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study examines the interaction between O_3, NO_2 and SO_2 and separately at low concentrations (0.15 ppm). A significantly enhanced decrease of specific airway conductance was observed during the exposure to O_3 + SO_2 in half of the subjects in comparison with O_3 alone. However there is considerable interindividual variability in the response to the pollutant exposure. It is not possible to discern from the present study when there is any additive effect. The number of subjects is small and the differences and means are also small. This has been show previously in other papers at higher concentrations (in this set of reviews). In terms of clinical relevance for SO_2, the concentration was too low to be of significance as the route of exposure was direct mouth inhalation. Only in asthmatic subjects are these concentrations of SO_2 relevant and the investigators should have examined this group.

Confidence index:

Authors provided an inadequate literature review in the introduction and an ambiguous objective statement. Wth regards to the materials and methodology section, a number of study flaws were evident. The number of subjects was small (n=7) and significant data would be hard to extract by statistics, especially when looking for subtle effects. Very little information was given for subjects involved; no occupational history was provided. Furthermore, it is unclear why the authors chose the concentration levels for each gas. O_3 is obviously more potent in eliciting bronchoconstriction compared to the other gases. By selecting 0.15 ppm for O_3, the effects at 0.15 NO_2 or SO_2 may be masked or overwhelmed by the O_3 response. A SO_2 concentration of 0.37 ppm may have been a better choice to compare with other studies cited by the authors. The study was too ambitious and more valuable information could have been gained by using only 2 gases and exploring a concentration gradient. In this way, a threshold may have been found instead of arbitrarily assigning a "lower" examination value (0.15 ppm).

Review – Confidence index ranking:

Moderate to low

Author: Kirkpatrick, M.B., Sheppard, D., Nadel, J.A., Boushey, H.A. | **Internal ID:** 074

Title: Effect of the oronasal breathing route on sulphur dioxide-induced bronchoconstriction in exercising asthmatic subjects

Year: 1982

Abstract We undertook a study to determine how the oronasal-breathing route affects the bronchoconstrictor response to sulphur dioxide (SO_2) inhaled by asthmatic subjects during exercise. In 6 subjects, we compared the changes in specific airway resistance (SRaw) caused by breathing humidified air through a mouthpiece during 5 min of exercise on a bicycle ergometer (550 kpm/min) to the changes caused by breathing humidified air plus 0.5 ppm of SO_2, (a) through a mouthpiece (oral breathing), (b) by facemask (oronasal breathing), and (c) by facemask with the mouth occluded (nasal breathing) during exercise. Breathing humidified air plus 0.5 ppm of SO_2 through a mouthpiece or by facemask during exercise significantly increased SRaw in all 6 subjects, and breathing humidified air plus 0.5 PPM of SO_2 by facemask with the mouth occluded significantly increased SRaw in 5 of 6 subjects. The increase in SRaw caused by breathing humidified air plus 0.5 PPM of SO_2 through a mouthpiece was not significantly different from the increase caused by breathing SO_2 by facemask (p greater than 0.05), but was significantly greater than the increase caused by breathing SO_2 by facemask with the mouth occluded (p less than 0.05). These results indicate that although nasal breathing partially protected against SO_2-induced bronchoconstriction in our subjects, both oral and oronasal breathing of low concentrations of SO_2 during exercise can cause significant bronchoconstriction in people with asthma.

Objective: The purpose of this study was to determine whether the bronchoconstrictor effects of low concentrations of SO_2 in asthmatic subjects vary during exercise when they breathe SO_2 by (a) mouthpiece (oral breathing), (b) facemask (oronasal breathing), or (c) facemask with the mouth occluded (nasal breathing).

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Humidified air + 0.5ppm SO_2	5 minutes	4 men 2 women	21-28 yrs	6	asthmatic	N/A	N/A

Observations:

Specific airway resistance was significantly increased in all 6 subjects after moderate exercise performed while breathing 0.5ppm SO_2 in humidified air either through a mouthpiece (oral breathing) or by facemask (oronasal breathing) and in 5 of the 6 subjects after breathing the same gas mixture from a facemask with the mouth occluded (nasal breathing).

The increase in specific airway resistance caused by breathing SO_2 through a mouthpiece was significantly greater than the increase caused by breathing humidified air through a mouthpiece ($p<0.01$).

The increases in specific airway resistance caused by breathing SO_2 either through a mouthpiece or by facemask were significantly greater than that caused by breathing humidified air through a mouthpiece, ($p<.01$ for breathing SO_2 by mouthpiece versus breathing air by mouthpiece, and $p<0.005$ for breathing SO_2 by facemask versus breathing air by mouthpiece).

He increase in specific airway resistance caused by breathing SO_2 through a facemask with the mouth occluded was significantly greater than that caused by breathing humidified air through a mouthpiece ($p<0.01$) but was significantly less than that caused by breathing SO_2 through a mouthpiece ($p<0.05$). For the group, the increase in specific airway resistance caused by exercising while breathing humidified air through a mouthpiece was significant ($p<0.05$) but very small. Sulphur dioxide dose-response curves constructed from data on 2 subjects indicated that the change in specific airways conductance increased as the concentration of SO_2 breathed during exercise increased, whether the SO_2 was breathed through a mouthpiece (oral breathing) or from a facemask (oronasal)
*Statistically significant

Author's Conclusions:

This study shows that exercising moderately while breathing 0.5ppm of SO_2 with humidified air through a mouthpiece, by facemask, or through the nose causes significantly more bronchoconstriction in asthmatic subjects we studied than exercising moderately while breathing humidified air through a mouthpiece.

Asthmatic subjects are at significant risk of developing bronchoconstriction when breathing even low concentrations of SO_2 during moderate exercise. Although nasal breathing appears to partially protect many asthmatic subjects from SO_2 induced bronchoconstriction, most will presumable switch to oronasal breathing during moderate and perhaps light, exercise, thus increasing their risk of SO_2 induced bronchoconstriction.

An unknown number of asthmatic subjects may breathe oronasally even at rest.

Therefore, we believe the results of this and previous studies that demonstrate the sensitivity of asthmatic subjects to the bronchoconstrictor effects of even low concentrations of SO_2 are relevant to the determination of air pollution standards.

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+/-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? (Possibly, financial remuneration) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+/-) Were subjects informed about the experimental risks? (No info) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels?

	(+)Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+/-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+/-)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+/-)Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+/-)Were the subjects randomly assigned to exposure groups? (+/-)Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+/-) Was a control group assayed? (+/-)Is the control regime appropriate? (-)Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (+/-)Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+/-)Were assessment measures sufficiently sensitive? (+/-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (+)Was individual data reported for each subject? (+/-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (-)Are confidence intervals reported?

	(+)Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+/-)Were assumptions reasonable? (+)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (+/-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

In this study, the effects of low concentration of SO_2 in asthmatic subjects vary during exercise when they breath SO_2 by (a)mouthpiece (oral breathing), (b) facemask (oronasal breathing), (c) facemask with the mouth occluded (nasal breathing) were examined. The point was to assess the ability of the nose and upper respiratory tract to protect people with hyper reactive airways from the bronchoconstrictor effects of SO_2 when inspiratory airflow is high, as it is during exercise. In this study, breathing nasally only partially protected the subjects from the bronchoconstrictor effects of SO_2. However, the SO_2 breathed nasally still caused significant bronchoconstriction in the subjects. This bronchoconstriction was significantly less than that caused by breathing orally and non-significantly less than SO_2 breathed oronasally. It also appeared from the results in this study that asthmatics may switch from nasal breathing to oronasal breathing during exercise.

There was interindividual variability in the responses, which makes the results difficult to compare. Dose-response curves for two subjects during exposure-exercise indicated that the change in specific airway resistance increased as the concentration of SO_2 breathed during exercise increased, whether the SO_2 was breathed through a mouthpiece (oral breathing) or from a facemask (oronasal breathing).

It is important to note the differences in response depending on the apparatus worn and depending on the individual. Many studies report consistency with other studies without pointing out that different breathing methods are used. Normal individuals and asthmatic individuals may breath differently depending on the level of exercise being performed. The degree or type of asthma may also affect the response in studies. For example, one subject in this study had a nasal condition which prevented nasal breathing.

This study maybe clinically relevant as it shows a protective effect when exposure is done through the nose to some extent (ie oronasal) although this result is not particularly novel.

Confidence index:

This study gave produced significant results that are biologically relevant. There are some inconsistencies regarding how the investigators determined some of the experimental conditions. The objective was to investigate the bronchoconstriction effects of SO_2 under different exposure pathways in asthmatics. The investigators chose to stress the subject through exercise. This was unnecessary as it has been previously shown that asthmatics are sensitive to 0.5 ppm SO_2 with mouth inhalation. Exercise may introduce a confounding factor as bronchoconstriction was shown in all subjects with exercise alone, although the difference was statistically insignificant in a few subjects. No control (non-asthmatic) group was tested which would have been useful for inter-group comparison. The test group was small (n=6) and included individuals with obvious physical abnormalities such as signs of allergy, deviated septum and nasal occlusion. Inclusion of the data from these individuals may introduce confounders and no correction was made for these individuals in the data analysis.

Review – Confidence index ranking:

Moderate to Low

Author: Frank, N.R., Amdur, M.O., Whittenberger, J.L.	**Internal ID:** 076

Title: A comparison of the acute effects of SO_2 administered alone or in combination with NaCl particles on the respiratory mechanics of healthy adults

Year: 1964

Abstract Changes in the pulmonary flow resistance (RI) of healthy male adults, induced by three levels of SO_2: 1-2, 4-6, and 14-7ppm, were compared when the gas was administered alone or in combination with submicronic sodium chloride aerosol. The subjects were seated in a volume-displacement body plethysmograph, breathing by mouth. At least one month transpired between exposures. No systematic differences were found between the responses to the gas alone and the gas-aerosol mixture. Essentially the same dose-response relations occurred in both circumstances: that is, the group showed no change in RI during exposure to 1-2ppm of SO_2, and showed a smaller increase to 4-6ppm than to 14-17 ppm. In a second type of experiment the gas and gas-aerosol mixture were administered one after the other, separated by a 15-min period of room-air breathing. The response to the second exposure whether it was the gas alone or the mixture-was smaller than the response to the first exposure.

Objective: Our purpose was to learn whether the acute changes in respiratory mechanics of healthy adults caused by SO_2 is intensified by the presence of submicronic NaCl particles as had been shown to occur in guinea-pigs.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Experiment 1 1. 1ppm SO_2 2. 4ppm SO_2 3. 15ppmSO_2 4. 1ppm SO_2 + NaCl aerosol 5. 4ppmSO_2+NaCl aerosol 6. 15ppmSO_2+aerosol	30 min each with minimum 1 month between exposures to SO_2 alone and SO_2+aerosol mixture	Male	25-56	12	healthy		
Experiment 2 Varied for each individual The first 7 experiments: 10 min exposure was with SO_2 and the second 10 minute exposure was with SO_2 + aerosol. The 7 experiments were performed a second time but with the exposures reversed.	14 experiments each consisting of : 10min exposure 15 min recovery 2nd 10 min exposure 15 minute recovery	Male		10			

Observations:

Experiments in which exposures ere carried out at least one month apart.

The average RI did not change significantly during exposure to the lowest level of SO_2, with or without particles added. There was an increase in mean RI for the group during exposure to the gas alone at both 5ppm (p<.001) and 15ppm (p<0.01), and an increase in RI during exposure to the mixture at 15 ppm (P<.001). Variations in the individual patterns of response were noted at the two higher levels of SO_2. No systematic differences ere found between the responses to the gas alone and to the gas-aerosol mixture. Generally, the increase in RI was maximal after 10 minutes of exposure.

Experiments in which the gas alone and gas-aerosol mixture followed one after the other
Changes in RI were greater during the initial exposure, whether the gas alone or the mixture was used, than during the second exposure 15 minutes afterwards (p<0.01).

*Statistically significant

Author's Conclusions:

"SO_2 caused an increase in RI in healthy subjects that was not systematically influenced by the addition of submicronic NaCl aerosol."

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+/-) Were subjects informed about the experimental risks? (No info) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? (+)Were the chosen exposure levels appropriate to investigate the primary objective? (+)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+)Was evidence provided to justify the dosing regime? (+/-)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined

	(-)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Were the subjects randomly assigned to exposure groups? (+/-)Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+)Is the control regime appropriate? (-)Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+/-)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (+)Was individual data reported for each subject? (+/-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+/-) Are the statistical methods used appropriate? (-)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (+)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables? (+/-)Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable?

	(+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the effect of NaCl particles and its effect on respiratory function in combination with SO_2 in healthy individuals. The results showed that the aerosol itself was not able to affect respiratory function and did not enhance SO_2 toxicity in combination. This study conflicts with previous studies performed in guinea pigs where NaCl does have a synergistic effect with SO_2. No explanation for the differences was given in the text. One important finding done as a secondary study showed that exposure of gas followed by gas-aerosol or vice-versa resulted in a second response that was smaller than the first. This shows that acute exposure causes a resistance mechanism to be invoked to protect the pulmonary system to secondary exposures.

Confidence index:

The number of subjects used in this study was adequate, however, little information about the subjects history was given. This gives rise to a number of potential confounding variables such as smokers, medication, and body size. The procedural and equipment of the study appeared to be adequately performed except for the omission of the relative humidity. This variable would seem to be very important in this study as NaCl is hygroscopic. From the experimental details and the discussion, the humidity would be expected to be high (>90%) yet an accurate and continuous reading would have helped in verifying the particle size of the aerosol, which was monitored. Also, the temperature was not disclosed. It is assumed that room temperature would be used, however, if this variable was not control, this would also have an effect on the aerosol evaporation and thus would alter effectiveness of the NaCl. The first experiment (exposure to SO_2 alone or SO_2+aerosol) was performed three times. However, each time the experiment was performed, it was only on a group of six subjects that differed for each of the three repetitions. Therefore, the three repetitions consisted of three different groups. The second experiment consisted of 14 experiments on 10 subjects. Seven subjects were exposed to SO_2 alone, recovered for 15 min, exposed to SO_2 + aerosol, and recovered for 15 min. The next seven experiments were performed on seven subjects with the exposures reversed. Therefore, the groups didn't match. Furthermore, i the second experiment, not only did the two groups of 7 differ, but also the concentrations of SO_2 differed for each individual. For example, in the exposure to SO_2 alone, the SO_2 concentration ranged from 9-19ppm. In the exposure to SO_2+NaCl, the concentration ranged from 10-21ppm.

There are additional results given for several subjects, who, as a result of participating in an earlier study, were exposed to similar levels of SO_2 alone or to the gas-particle combination at least twice. However, there is no other information given re: methods, etc. In addition, there wasn't enough detail regarding the statistical analysis.

Review – Confidence index ranking:

Low

Author: Kehrl, H.R., Roger, L.J., Hazucha, M.J., Horstman, D.H. | **Internal ID:** 078

Title: Differing response of asthmatics to sulphur dioxide exposure with continuous and intermittent exercise

Year: 1987

Abstract Ten subjects with mild asthma were initially exposed in an environmental chamber (26 degrees C 70% relative humidity) to clean air and 1.0 ppm SO_2 while performing 3 sets of 10-min treadmill exercises (ventilation, 41 L/min) broken by 15-min rest periods. To evaluate the effects of the pattern and duration of exercise on the response to SO_2 exposure, the subjects were then exposed to the same environmental conditions while exercising continuously for 30 min. Specific airway resistance (SRaw) was measured by body plethysmography before each exposure and after each exercise. All SO_2 responses were significantly greater than the clean air responses. With intermittent exercise and SO_2 exposure, mean SRaw measurements (preexposure and after 10, 20, and 30 min of exercise) were 5.4, 14.7, 12.8, and 11.1 cm H2O/s. After SO_2 exposure with continuous exercise, the mean SRaw showed an increase from 5.2 to 17.3 cm H_2O/s. This increase was significantly ($p = 0.018$) greater than the response after the third exercise in the intermittent protocol. It appears that asthmatics show an attenuated response to repetitive exercise in an atmosphere of 1.00 ppm SO_2 and that the response to SO_2 exposure develops rapidly and is maintained during 30 min of continuous exercise.

Objective: To examine the response of asthmatics exposed to 1ppm SO_2 while continuously exercising for 30 min and compared these responses with those previously observed for the same subjects performing 3 10min exercises separated by 15min periods of response testing and rest.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Exp. 1 1. clean air 2. 1ppm SO_2	Subjects performed 3 10-min cycles of exercise, separated by 15-min of testing and rest. Exercise intervals completed at 10,35,60-min exposure.	male	20-33yrs	10	asthmatic	unknown	unknown
Exp. 2 1. clean air 2. 1ppm SO_2	Subjects exercised for 30-min continuous.						

Observations:

Specific airway resistance (SRaw) was measured by body plethysmography before each exposure and after each exercise. All SO_2 responses were significantly greater than the clean air responses. With intermittent exercise and SO_2 exposure, mean SRaw measurements (pre-exposure and after 10, 20 and 30 min of exercise) were 5.4, 14.7, 12.8, and 11.1 cm H_2O/s. This increase was significantly ($p=0.018$) greater than the response after the third exercise in the intermittent protocol.

Only 4 subjects clearly had an attenuated response after repeated exercise in SO_2. However, the magnitude of the decline in response of these 4 subjects was sufficient to affect the group mean SRaw and compared with that in the first exercise, the SO_2 response was progressively reduced after the second and third exercises.

The increase in mean SRaw induced by SO_2 exposure with the subjects performing 30 min of continuous exercise was greater than the changes after the first, second, and third exercise intervals during the SO_2 exposure with intermittent exercise.

Compared with clean air, 30 min of continuous exercise in 1ppm SO_2 resulted in small but significant decrements in other pulmonary function measurements.

The decreases observed in the spirometry performance after exposure to 1ppm SO_2 were about the same with the 2 (continuous vs. intermittent) exercise conditions.

*Statistically significant

Author's Conclusions:

The magnitude of bronchoconstriction in subjects with mild, methacholine-sensitive asthma declines upon repeated 10-min exercise intervals in an SO_2 atmosphere.

Our observations suggest that the attenuated response of some asthmatics to repetitive SO_2 challenges is likely a function of mechanisms that are not only active during the exercise but also during the intervening rest or recovery periods.

From the present knowledge of SO_2-induced bronchoconstriction of moderately ventilating asthmatics, it is apparent that the response develops rapidly and is maintained or possibly even increases when high ventilation rates are maintained during the SO_2 exposure. When the exercise is interrupted for a sufficient time period, there is an attenuation of the response to a subsequent exercise in a SO_2 atmosphere.

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (+) Was the method of recruitment appropriate? (No Info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? (No info) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels?

	(+)Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Were the subjects randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+)Is the control regime appropriate? (+)Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (+)Was individual data reported for each subject? (N/A)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported?

	(+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (+/-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

After repetitive exercise, the asthmatics in this study showed an attenuated response in an atmosphere of 1ppm SO_2. With continuous exercise, it was determined that the response to SO_2 exercise develops rapidly and is maintained during 30 min of continuous exercise. The subjects were reported as having only mild asthma and the results may have differed for more extreme cases. These results indicate that the response of 'mild' asthmatics to SO_2 exposure may depend more on the type of exercise being performed and not so much on the total dose, and that there may be a protection mechanism partially protecting the subjects. However, the amount of attenuation may depend on the rest interval between exposures.

This result is important clinically as it gives insight into the mechanism of SO_2 induced bronchoconstriction. The authors cite similar experiments with other stimuli such as cold, dry air that give comparable results. This would imply that the two stimuli share similar mechanisms.

Confidence index:

There are a number of shortcomings in the study. The intermittent exercise, because of the rest period, would likely decrease total ventilation rates compared to continuous exercise. This translates into a lower dose of SO_2, which may explain the decrease in bronchoconstriction. The statistical design of the study was also lacking. No tests for significance were given between test conditions. Differences were compared by using absolute averages with standard deviation. The number of subjects was marginal (n=10) to produce significant data although they were thoroughly screened to limit confounders. Furthermore, the subjects were all young, white males, making extrapolation of results to other populations uncertain. Furthermore, the subjects were recruited from a larger study. Asthmatics who had previously been shown to be either insensitive or very sensitive to the effects of SO_2 exposure were not included. This mode of selection may have introduced some selection bias.

Though the increases were not significant, there were increases in SRaw during exercise in clean air which may have been due to exercise induced bronchoconstriction.

Review – Confidence index ranking:

Moderate

Author: Wolff, R.K., Obminski, G., Newhouse, M.T. | **Internal ID:** 084

Title: Acute exposure of symptomatic steelworkers to sulphur dioxide and carbon dust: effects on mucociliary transport, pulmonary function, and bronchial reactivity

Year: 1984

Abstract Nine steelworkers participated in controlled exposures to sulphur dioxide alone and sulphur dioxide plus carbon dust (5 ppm and 10 mg/m^3, respectively). All were experiencing work related respiratory difficulties. Bronchial clearance was measured using radioaerosol inhalations and external detection. Results were variable and no statistically significant changes were observed. One asthmatic showed a complete cessation of clearance during exposure to sulphur dioxide and carbon dust. Bronchial reactivity was found to be significantly raised after exposure to sulphur dioxide but equivocal results were found after exposure to sulphur dioxide and carbon dust. Pronounced changes in pulmonary function were seen only in the two asthmatic subjects. They could not tolerate the levels, indicating that these threshold limit values are too high, at least for these individuals who showed much greater sensitivity to the pollutants than the others.

Objective: To determine whether symptomatic steelworkers showed responses in mucociliary clearance and pulmonary function similar to the group of healthy volunteers previously studied or whether they exhibited any altered sensitivity. To see if there were correlations between changes in different measurements and if any one measurement was more sensitive than the others were.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
1. placebo 2. 5 ppm SO_2 3. 5 ppm SO_2 + 10 mg/m^3 carbon dust	2.5 hrs each	male	26-62	9	Respiratory difficulties	unknown	unknown

Observations:

The changes in pulmonary functions were not dramatic except for the exposures of subjects 8 and 9, both asthmatics.

The results of clearance were mixed with both speeding and slowing of clearance.

The greatest change in bronchial reactivity occurred on exposure to SO_2, when virtually all subjects had a much greater fall in FEV_1 with the same histamine concentration compared with the exposures to placebos. Changes were significant during exposure to SO_2 ($p<0.05$) but were not significant during exposure to SO_2 plus carbon dust (p.05).

*Statistically significant

Author's Conclusions:

Overall, the results indicate that individuals wit a degree of respiratory disease have more variable responses than healthy subjects. It is clear that asthmatics appear to be at particular risk and their exposure to irritant gases and particles should be limited.

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (+) Was the method of recruitment appropriate? (No info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? (Not enough info Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? (+)Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+)Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Were the subjects randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+)Is the control regime appropriate? (-)Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale?

	(+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (+)Was individual data reported for each subject? (+)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+/-)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study examined the TLV for sulphur dioxide and how it affected workers already suffering from some form of workplace respiratory distress. As such, it provides some support for human exposure guidelines, especially in regards to sensitive populations that the TLV for occupational exposures is not appropriate for the general population. However, There were no statistically significant changes in any of the pulmonary function measurements for the set of subjects as a whole, and no changes which were seen after exposures to pollutant occurred only in the two asthmatics. The subjects in this study did not represent a homogenous group; and so it is difficult to generalize from this data. The responses were too variable.

Confidence index:

It is unclear what the objective of this study was. Subject screening was not selective and all had symptoms of respiratory difficulties. Two of the subjects were asthmatics. No other history except for work and respiratory history were given. If the data were examined on the whole, confounders would have made the data inconclusive. However, the data was presented for individuals and there seem to be a focus on the pulmonary functions and symptoms of the two asthmatic subjects. The non-asthmatic subjects appear to behave as "normal" subjects with little response to the SO_2 concentrations in question. The exposure procedure was flawed in that carbon dust was not given alone. This data would have allowed the investigation of synergistic effects of SO_2 and carbon dust. It is unclear if any concrete conclusion can be made from this study. The number asthmatic subjects are too small to give a complete picture. At best, this study gives some striking anecdotal evidence about asthmatic response to SO_2.

Review – Confidence index ranking:

Low

Author: Yang, S.C., Yang, S.P.	**Internal ID:** 086
Title:Respiratory Function Changes from Inhalation of Polluted Air	
Year: 1994	
Abstract Exposure to polluted air may trigger respiratory symptoms in certain subjects. Maximal expiratory flow volume measurements and methacholine bronchial challenges were conducted in 25 asthmatics and 30 healthy volunteers, before and after breathing bagged polluted air for 30 min, to determine whether exposures to polluted air influence respiratory function and bronchial reactivity. No differences in forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1.0), total respiratory resistance (Rrs), and bronchial reactivity were noted for normal subjects after they inhaled polluted air and ambient air. Significant decreases in FEV1.0 and FEV1.0/FVC, an increase in airway reactivity, and elevation of baseline Rrs values occurred in the asthmatic subjects, after exposure to polluted air. These findings suggest that asthmatic patients are more vulnerable to the adverse effects of polluted air.	
Objective: The purpose of this study was to demonstrate whether short-term (30min) exposures to polluted air influence lung function or increase bronchial sensitivity to methacholine challenge in normal subjects and in asthmatic subjects.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Polluted Air: SO_2 112±19 ppb	30 minutes	15 men 10 women	23-48yrs	25	Asthmatic	N/A	N/A
		16 men 14 women	20-45 yrs	30 (control)	Healthy		

Observations:

Exposure to polluted tunnel air did not significantly affect pulmonary functions in healthy individuals.

In the asthmatic group, a significant decrease in $FEV_{1.0}$ ($p<.05$) and increase in Rrs (total respiratory resistance) ($p<.001$) were observed after inhalation of polluted air.

There was also a significant reduction in the $FEV_{1.0}$(forced expiratory volume in 1 s)/FVC (forced vital capacity) ratio.

In the asthmatic group, methacholine challenge performed after inhalation of polluted air resulted in a significantly higher baseline Rrs value than those measured prior to exposure.

*Statistically significant

Author's Conclusions:

In summary, we conclude that inhalation of polluted tunnel air by this group of asthmatic subjects led to significant changes in pulmonary function.

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+)Are the subjects a representative sample of the population to which extrapolations will be made? (+) Was the method of recruitment appropriate? (No info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? (No info) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? (+)Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (Yes)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+)Was evidence provided to justify the dosing regime? (+/-)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Were the subjects randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+)Is the control regime appropriate? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom)

	(+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (+/-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (-)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study examines respiratory function changes from the inhalation of polluted air in a group of asthmatics and a group of healthy individuals.

SO_2 was measured in the pollution samples. The effects of SO_2 alone cannot be determined from this study, however, this wasn't the objective of the study. There were some significant changes in pulmonary functions in the asthmatic subjects. The responses of the asthmatic subjects were not compared to those of the normal controls, so the differences in effects between these two groups cannot be examined. The findings in this study suggest that air pollution aggravates respiratory symptoms in asthmatic patients. The biological findings are not surprising considering the route of exposure (mouth) and the high concentrations of the other gases.

Confidence index:

In general, the study design was adequate to look at pollution as a mixture but conclusions are difficult to verify in terms of a single gas. The number of subjects was relatively high with a group of "normal" subject to compare with the asthmatics and a full medical workup was done, however, there was no description on the screening of subjects and the protocol for rejection. There was some control of confounders such as restriction of medication but there was no mention of exclusion of smokers or atopics. The exposure of the subjects to polluted air is interesting, however, there was no comparison to pure gases alone. This would make the data interpretation difficult, as the impact of the gases along with synergistic interactions cannot be measured. Also, the investigators failed to measure

the ozone in the polluted air, a powerful irritant that is likely present in significant concentrations in polluted air. Also, the humidity and temperature was not given, measured or monitored. These variables are important to the study of polluted air that was the objective of the study, yet neglected by the authors.

Review – Confidence index ranking:

Moderate

Author: Sandstrom, T., Stjernberg, N., Andersson, M.C., Kolmodin-Hedman, B., Lundgren, R., Rosenhall, L., Angstrom, T.

Internal ID: 090

Title: Cell response in bronchoalveolar lavage fluid after exposure to sulphur dioxide: a time-response study

Year: 1989

Abstract: Bronchoalveolar lavage (BAL) has been performed in 22 healthy nonsmoking male volunteers after exposure to 8 ppm SO_2 (20 mg/m^3). The exposure level exceeds the US Short-Term Exposure Limit (STEL) of 5 ppm, but occurs as peak exposures in industrial indoor environments. Exposures were made during light work on a bicycle ergometer in an environmental exposure chamber for 20 min. BAL was performed 2 wk or more before exposure and 4, 8, 24, and 72 h after exposure in eight subjects at each time interval. Four hours after exposure significant increases were found in the numbers of lysozyme-positive macrophages, lymphocytes, and mast cells (p less than 0.02 to 0.05). Lymphocytes, lysozyme-positive macrophages, total count of alveolar macrophages, and total cell number increased to peak values 24 h after exposure (p less than 0.02 to 0.05). Seventy-two hours after exposure the cell numbers and distribution had returned to normal. The time course of the cell reactions found in BAL fluid after controlled SO_2 exposure represents a new and previously not reported response to a noxious gas.

Objective: Our aim, in this study, was to use BAL (bronchoalveolar lavage) technique to determine the time course of the cell reactions in the human lung after SO_2 exposure.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
8 ppm SO_2	20 min (exercise performed during last 15 min)	Male	22-37 yrs	22	healthy	N/A	N/A

Observations:

A significant increase in total cell number was found 8 h after exposure and reached a peak value at 24 h. The increase consisted of alveolar macrophages/monocytes, lymphocytes, and mast cells, whereas eosinophils and neutrophils were unaffected.

The total count of lymphocytes was significantly increased 4 h after exposure and reached peak values at 24 h, when an additional increase in the relative amount of lymphocytes was found.

The increase in the total amount of macrophages at 8 h and 24 h correlated with the increase in the total amount of Lysozyme-positive macrophages ($r=.69$, $p<0.001$).

A significant increase in the total and relative count of mast cells was seen 4 h after exposure.

In summary, exposure to SO_2 increased mast cells, alveolar macrophages and lymphocytes in the recovered BAL fluid.

*Statistically significant

Author's Conclusions:

We believe that SO_2 exposure caused a monophasic mastocytosis that lasted 4 to 24h after exposure.

We do not feel that the results conclude that sulphur dioxide exposure induces a true biphasic mast cell migration to the air spaces.

We believe that the pulmonary response to SO_2 is more pronounced in terms of acute bronchitis/bronchiolitis than alveolitis.

It is concluded that alveolar macrophages, lymphocytes, and mast cells are all involved in the inflammatory cell response 4h after brief exposure with 8 ppm SO_2.

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (+) Was the method of recruitment appropriate? (No info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? (No info) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? (+)Were the chosen exposure levels appropriate to investigate the primary objective? (+)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+/-)Were the subjects randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+)Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)

E. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (+/-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (+)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The authors examined the time course of cell reactions found in BAL fluid after controlled SO_2 exposure. After 4 h and 24 h, significant increases occurred in the numbers of lysozyme-positive macrophages, lymphocytes, and mast cells. However, there was no increase after 8 h, indicating insufficient statistical power or a possible problem with the experimental procedures. The mechanisms behind this cell response to SO_2 still need to be clarified. The clinical importance of this paper is that this technique is a direct measure of the biological response due to SO_2 exposure and could be useful to support lung function data.

Confidence index:

The use of BAL is interesting, however, there are some inherent difficulties associated with this and other bioassays of this type. Individual differences are very large no matter how stringent the screening method for the test subjects. This is shown in the data where there are large variations within each test group. The study design was not appropriate because of this. The test pool was split into four and each subject exposed once. To limit variability, each subject could be tested after exposure to each condition. This would allow comparison between exposure conditions. Also, there was no "placebo" exposure. Although the chance of exercise inducing the observed effects is small, the investigator should have tested this to limit confounders. The use of a time course to study the effect is interesting yet introduces confounders as the time between testing is large.

Significant increases in cells in the BAL were detected after 4h and 24h, but not at 8h. The sample size may not have been large enough to detect a significant increase.

There were 22 subjects who provided control data and who were exposed to the SO_2. However, the data for 4, 8, 24, and 72 hours after exposure only include n=8. There is no information on how the 8 subjects were chosen, or if the same 8 subjects are compared, etc. There is no information on why data for n=22 was not included.

There is no information on work history, medical history, other chemical exposures etc. for the subjects. BAL fluid was examined 4,8,24, and 72 h after exposure and other chemical exposures may confound results. The control values for BAL examinations were performed 2wks before the exposure. There is no justification for this timeframe.

Review – Confidence index ranking:

Low

DRAFT

Author: Sandstrom T. Stjernberg N. Andersson MC. Kolmodin-Hedman B. Lundgren R. Rosenhall L. Angstrom T. **Internal ID: 091**

Title: Cell response in bronchoalveolar lavage fluid after exposure to sulfur dioxide: a time-response study.

Year: 1989

Abstract: Environmental chamber exposure and bronchoalveolar lavage (BAL) were used to study the dose-response relationship between short-term exposure to sulfur dioxide (SO_2) and inflammatory reactions in the human lung as reflected in BAL fluid. Health subjects were exposed to 10, 13, 20, or 30 mg/m^3 for 20 minutes. BAL was performs several weeks pre-exposure and 24 h postexposure. Mast cells, lymphocytes, lysozyme positive macrophages, and the total number of macrophages were significantly increased after SO_2 exposure. A dose-dependent increase in the cell response in BAL fluid was observed after exposure to 10-20 mg/m^3, but no further increase was detected after 30 mg/m^3. Inflammatory cell response was found in BAL fluid at SO_2 levels that occur in industrial indoor environments worldwide, and cell response to SO_2 was also seen below the short-term exposure limit of Sweden and many other countries (13 mg/m^3).

Objective: "… we have performed the present study to determine the exposure dose-response relationship between short-term exposure to SO_2 and the inflammatory response in the human lung."

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
10-30mg/m^3 (4-11ppm)	**20min**	**Male**	**22-37**	**22**	**Healthy nonsmoking**	**n/a**	**10mg/m^3**

Observations:

"All the subjects had normal endobronchial findings in the reference bronchoscopy before exposure and after exposure to SO2 concentrations of 10 and 13mg/m^3 (4 and 5ppm, respectively). Twenty-four hours after exposure to 20mg/m^3 (8ppm) mucosal erythema was found in the distal half of the trachea and proximal main bronchi of all the subjects. The erythema was even more pronounced 24h after exposure to 30mg/m^3."

"A significant increase in the total number of cells was found in the BAL fluid 24h after exposure to 20 and 30mg/m^3 (8 and 11ppm, respectively). The increase consisted of lymphocytes, alveolar macrogphages/monocytes, and mast cells, while the eosinophil and neutrophil levels were unaffected."

"Lysozyme positive macrophages/monocytes were significantly increased at all the examined concentrations. The total number of alveolar macrophages/monocytes were significantly increased at all concentrations. The total number of alveolar macrophages/monocytes was significantly increased 24h after exposure to 20 and 30mg/m^3 (8 and 11ppm, respectively."

Increases were also seen in the total number of mast cells 24h after exposure to 13mg/m^3 SO_2, and peaked with the 30mg/m^3 exposure (5 times higher than pre-exposure).

The numbers of lymphocytes was also significantly increased following 20 and 30mg/m^3 SO_2 exposure.

*Statistically significant

Author's Conclusions:

"The cytologic examination of the recovered bronchoalveolar lavage fluid showed that SO_2 exposure gives an inflammatory cell reaction involving mast cells and alveolar macrophages even at the relatively low SO_2 concentrations of 10-13mg/m^3 (4-5ppm). The macrophage reaction was more pronounced in terms of increased lysozyme production in the cells, visible by specific staining, than the increase in the total number of macrophages."

"We conclude that the inflammatory cell response that was found in the BAL fluid after SO_2 exposure increased as the dose increased from 10-20mg/m^3, but that no further amplification of the reaction could be detected after 30mg/m^3."

A. Subjects:	+✏ Is the number of subjects sufficient to produce statistically significant results? ±✏ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ±✏ Are the subjects a representative sample of the population to which extrapolations will be made? +✏ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +✏ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	±✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✏ Was there a gradient of exposure levels? +✏ Were the chosen exposure levels appropriate to investigate the primary objective? +✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? +✏ Was evidence provided to justify the dosing regime? +✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✏ Was a full description of the monitoring devices provided? +/-✏ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✏ Were the subjects randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✏ Was a control group assayed? -✏ Is the control regime appropriate? +✏ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✏ Was the length of follow-up appropriate? +✏ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical?

	-✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? -✎ Did the investigator appropriately interpret the statistics? -✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The BAL technique was used to evaluate the dose-response relationship exposure of SO_2. The results showed that all the subjects had normal endobronchial findings in the reference BAL and after exposure to 4 an 5 ppm SO_2. 24 hours after exposure to 8 ppm SO_2, mucosal erythema was found.

A significant increase in the number of cells was found in BAL fluid 24 hours after exposure to 8 and 11 ppm SO_2. Lysozyme positive macrophages were significantly increased at all the examined concentrations. The total number of alveolar macrophages was significantly increased 24 hours after exposure to 8 and 11 ppm. The results showed that macrophage and lymphocyte response to SO_2 is non-linear with a declining slope above 8 ppm and an inflammatory response was found in the BAL fluid at SO_2 exposures less than the short-term exposure limit for Sweden. The clinical significance of this paper is that direct evidence of SO_2 exposure can be quantified in a dose-response manner

Confidence index:

This study is a variation of study number 090 and thus has the same inherent shortcomings stemming from a biological assay. The use of BAL is interesting, however, there are some inherent difficulties associated with this and other bioassays of this type. Individual differences are very large no matter how stringent the screening method for the test subjects. This is shown in the data where there are large variations within each test group. The study design was not appropriate because of this. The test pool was split into 4 and each subject exposed once. To limit variability, each subject could be tested after exposure to each condition. This would allow comparison between exposure conditions. Also, there was no "placebo" exposure. Although the chance of exercise inducing the observed effects is small, the investigator should have tested this to limit confounders. The text did not state if the subjects were isolated after exposure as any activity by subjects after the exposure may change the distribution/number of the cells prior to BAL. Simply walking out into a cold environment maybe enough to induce the observed effects. The statistical analysis was not complete. The data was given as an average with a range, however, a standard deviation would have been more useful to evaluate the variability of the data.

Review – Confidence index ranking:

Low to Moderate

DRAFT

Author: Tan, W.C., Cripps, E., Douglas, N., Sudlow, M.F.	**Internal ID:** 092

Title: Protective effect of drugs on bronchoconstriction induced by sulphur dioxide

Year: 1982

Abstract

The response to inhaled sulphur dioxide in eight normal, seven atopic, and 22 asthmatic subjects was studied by measuring thoracic gas volume and airway resistance in a whole-body plethysmograph. The fall in specific airway conductance in relation to the concentration of sulphur dioxide inhaled (0-20 ppm) was determined in all three groups. The specific airway conductance fell significantly in the atopic and asthmatic subjects but not in the normal group. In a double-blind study prior inhalation of disodium cromoglycate caused a significant reduction in the response to sulphur dioxide inhalation in atopic and asthmatic subjects. Prior treatment with inhaled ipratropium bromide blocked the response in the atopic subjects, but the effect was variable in the patients with asthma. Previous treatment with inhaled clemastine also reduced the response in patients with asthma, without causing a change in baseline specific conductance. We conclude that non-allergic bronchial hyperreactivity was increased in the atopic and the asthmatic subjects and that mediator release, in addition to a vagal reflex, has a role in such bronchoconstriction.

Objective: To examine the protective effects of drugs on bronchoconstriction induced by sulphur dioxide, in normal subjects, atopic non-asthmatic subjects, and patients with asthma. The changes in bronchomotor tone induced by sulphur dioxide may result from a vagal reflex or from mediator release from mast cells. To investigate this further we measured the influence of an anticholinergic drug (ipratropium bromide), disodium cromoglycate, and an antihistamine drug (clemastine) on bronchoconstriction induced by sulphur dioxide.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Study 1: 2.5-20ppm In all subsequent studies the subject inhaled the concentration of SO_2 that had previously given wither the greatest fall or at least a 30% fall in specific airway conductance (sGaw). Healthy: 20 ppm Atopic: 20ppm Asthmatic: 10 ppm	**Study 1:** 5 min at each concentration	**5 men** **3 women**	**20-42 yrs**	**8**	**Healthy**	N/A	N/A
		5 men **2 women**	**29-42 yrs**	7	**Atopic**		
		14 men **8 women**	**16-60yrs**	**22**	**Asthmatic**		
				7	**Atopic**		

Study 2: Ipratropium bromide administered before exposure at concentrations above **Study 3:** Placebo or disodium cromoglycate administered before exposure at concentrations above **Study 4:** 0.9% saline or clemastine administered before exposure				**9** **6** **5** **18** **7**	**Asthmatic** **Normal** **Atopic** **Asthmatic** **Asthmatic**		

Observations:

The maximal fall in sGaw occurred within five minutes of the inhalation of sulphur dioxide in all the subjects studied.
At 10 ppm and 20 ppm the mean percentage falls in sGaw in the atopic subjects were significantly greater than those in the normal subjects ($p<0.01$). At 10 ppm the percentage fall in the asthmatic patients was also significantly greater than in the normal subjects ($p<0.001$).

Effect of ipratropium Bromide on the Response to Sulphur Dioxide Inhalation

After inhaling ipratropium bromide the mean baseline sGaw in the nine asthmatic subjects increased significantly ($p<0.01$). In the asthmatic patients the individual responses to 10 ppm were variable, but the mean fall in sGaw after sulphur dioxide inhalation was significantly reduced.

Effect of Disodium Cromoglycate on Response to Sulphur Dioxide Inhalation

The falls in sGaw after inhalation of sulphur dioxide were significant in the atopic and asthmatic subjects ($p<.01$), but not in the normal subjects.

Effect of Clemastine on the Response to Sulphur Dioxide Inhalation

In seven asthmatic patients, the fall in sGaw produced by 10-ppm sulphur dioxide after inhalation of clemastine was significantly less than the fall after the inhalation of the saline.

*Statistically significant

Author's Conclusions:

We conclude that in addition to the vagal reflex mediator release from mast cells has a role in bronchoconstriction induced by sulphur dioxide. This may be initiated directly by sulphur dioxide or after the release of histamine or other mediators that have been shown to stimulate irritant receptors in animals.

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? (No info)Would the participation incentive employed cause the selection of an unrepresentative sample population? (+/-) Were subjects informed about the experimental risks? (No info) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? (+)Were the chosen exposure levels appropriate to investigate the primary objective? (+)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+/-)Was the duration of exposure appropriate to investigate the primary objective? (-)Was the duration of exposure precisely defined? (+/-)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+/-)Was evidence provided to justify the dosing regime? (+/-)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Were the subjects randomly assigned to exposure groups? (+/-)Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+)Is the control regime appropriate? (+)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc)

	(+)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (+/-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+/-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-)Did qualified personnel conduct the exams? (+/-)Was the timing and frequency of assessment logical? (+/-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (+/-)Was individual data reported for each subject? (N/A)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+/-)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The study focuses on the protective effect of some commonly used drugs on exposure to SO_2 in asthmatics, atopics and normals. The specific airway conductance fell significantly in the atopic and asthmatic subjects but not in the normal group after 20 minutes exposure to sulphur dioxide.

The protective effects of drugs on bronchoconstriction varied. For disodium cromoglycate, a significant protective effect appeared to occur for the atopic and asthmatic group, but not the normal group. This suggests a similar mechanism for both asthmatics and atopics. The ipratropium bromide blocked the response in the atopic subjects. The data for the asthmatic group that inhaled the ipratropium bromide varied among the individuals with asthma. Clemastine reduced the response to SO_2 exposure in asthmatics. This drug was not tested on the normals or the atopics.

The results confirm those done previously with the same drugs. The clinical impact of this paper is that these drugs serve an important therapeutic role for atopics and asthmatics for SO2 exposure and pre-exposure. Also, the effect of these drugs in different test groups suggests the mechanism of SO_2 bronchoconstriction.

Confidence index:

Information about the subjects in this paper are limited. The normal group was declared healthy yet not criteria was given for screening. Other important factors such as smoking, drug use, work history was not given leading to the distinct possibility of confounders. The age range of the asthmatics was large (16-60) which will have a large impact in the variability in the responses in that test group. By contrast, the age ranges for the normal and atopic groups were 20-42 years and 29-42 years old respectively.

The experimental procedure was not given in detail. There was no indication about the route of exposure except that it was probably nasal or oronasal due to the high concentrations the asthmatics were exposed to. The SO_2 exposure conditions such as humidity, temperature and even exposure time were not given. There was no indication about the timing of the measurements after the exposure.

It is apparent that the focus of the study was not about the toxicological impact of SO_2 but the drugs that protect against bronchoconstriction. The SO_2 concentration was determined by preliminary trials to give a 30% fall in sGaw. Furthermore, the first study consisted of exposing all of the subjects to varying concentrations of SO_2 to obtain dose-response curves, but this data was not included in the study.

Review – Confidence index ranking:

Low

Author: Witek TJ Jr. Schachter EN. Beck GJ. Cain WS. Colice G. Leaderer BP.	**Internal ID: 093**
Title: Respiratory symptoms associated with sulfur dioxide exposure.	
Year: 1985	
Abstract: Exposures to sulfur dioxide (SO_2) have been associated with progressive, dose-dependent bronchoconstriction in sensitive individuals. The clinical significance of such changes remains poorly characterized. We studied subjective responses following exposure to low level concentrations of SO_2 (less than 1 ppm) in a group of 10 healthy and 10 asthmatic subjects. The number and severity of complaints associated with SO_2 increased with concentrations in both healthy and asthmatic subjects. Asthmatics indicated progressive lower respiratory complaints, such as wheezing, chest tightness, dyspnea and cough with increasing levels of SO_2 while healthy subjects complained more frequently of upper airway complaints such as taste and odor with increasing levels of SO_2. Exercise increased the frequency of lower airway symptoms in asthmatics but led to no increases in symptoms in healthy subjects.	
Objective: "This report deals with the pattern of subjective complaints in both asthmatic and non-asthmatic subjects following exposures to low levels of SO_2, and seeks to determine if these subjective responses relate to lung function changes."	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0-1.0ppm	**40min**	**n/a**	**a) 26±6.3** **b) 27±5.1**	**20**	**a) 10 healthy** **b) 10 asthmatic**	**n/a**	**0.25ppm**

Observations:

"Pulmonary function testing revealed that statistically significant decrements in most lung function parameters were generally confined to concentrations of 0.75ppm and greater for the asthmatics engaged in exercise. No exercise-induced bronchospasm (EIB) was detected when the asthmatics exercised in the absence of SO_2."

"The number of individual complaints, totaled for all symptoms, as well as the total of the scaled complaint score increased with the concentration of SO_2."

"The number of selected respiratory complaints and the sum of the scaled symptom scores for lower airway symptoms in our asthmatic subjects were at least twice as great when exposure to 1.0ppm was accompanied by exercise as compared to the non-exercise exposure to 1.0ppm. These differences between rest and exercise were not seen for the non-asthmatics."

"The number of complaints and symptom scores for taste and odor were twice as frequent in healthy subjects as in asthmatics, both at rest and with exercise at exposures of 1.0ppm, while symptoms of dyspnea and chest tightness were only reported by asthmatics throughout the experiment."

*Statistically significant

Author's Conclusions:

"In conclusion, we have demonstrated that respiratory symptoms during and following low-level SO_2 exposures with and without exercise are mild and short-lived, but do increase with increasing SO_2 levels. Symptoms related to lower airway dysfunction such as dyspnea and chest tightness were confined to asthmatics while healthy subjects complained frequently of unusual taste and odor. These findings suggest that subjective symptoms during air pollution exposure may provide a guide to assess the clinical importance of the health effects of SO_2."

Review – Assessment:

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ±✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? ±✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ±✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined ±✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? +✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams?

	+✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? ±✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigates if subjective evaluation is useful for evaluating effects from SO_2 exposure. The results show that low levels of SO_2 show small, short-lived effects in asthmatics. This study is clinically relevant as it outlines a simple, inexpensive method for evaluation of SO_2 effects. Furthermore, separation of upper and lower airway effect can be performed without invasive tests (such as bronchoaveolar lavage).

Confidence index:

To assess the utility of the subjective evaluation, the study should have also measured quantitative endopoints such as FVC, SRaw, and other indicators of bronchoconstriction. As the authors pointed out, the effects of low-level SO_2 were small even with exercise in asthmatics. Some of the more subtle effects may have been missed by this technique and for full confidence, quantitative measurements need to be compared. The number of subjects was adequate, however, the medical and work histories were missing. Confounding variables such as smokers, medication use by the asthmatics, and respiratory ailments were possible. The experimental design was hard to critique as a reference for the monitoring and exposure was not given. Although the data is believable considering previous studies, the lack of information causes the technique to be questionable for sensitive measurements.

Review – Confidence index ranking:

Moderate

Author: Kulle, T.J., Sauder, L.R., Hebel, J.R., Miller, W.R., Green, D.J., Shanty, F.	**Internal ID:** 096
Title: Pulmonary Effects of sulfur dioxide and respirable carbon aerosol.	
Year: 1986	

Abstract

Four-hour individual and combined exposures to 1.0 ppm sulfur dioxide (SO_2) and 0.5 mg/m^3 (1.5 micron mass median diameter) activated carbon aerosol (ACA) were studied in 20 healthy nonsmoking subjects to determine if activated carbon as a "carrier" aerosol can augment the pulmonary response to SO_2. Fifteen-minute exercise stints (VE = 35 liters/min) were performed at commencement and completion of each 4-hr period. Significant increases in nose or throat irritation occurred with the SO_2 and SO_2 + ACA exposures and in eye irritation with the SO_2 + ACA exposure. Small, statistically significant decrements in spirometric function were observed following the first exercise (t = 17 min) for both the SO_2 and SO_2 plus ACA exposures; no significant changes were associated with the ACA exposure. Comparing function changes between the SO_2 and the SO_2 + ACA exposures demonstrated no statistically significant differences, thus a lack of SO_2 response enhancement by the carbon aerosol. The determination that the activated carbon sorbed only 1% of the SO_2 challenge concentration could explain the observed lack of SO_2 response enhancement.

Objective: To examine pulmonary function and bronchial challenge response to 1 ppm SO_2 and 0.5 mg/m^3, 1.5µm activated carbon aerosol (ACA).

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Day 1: filtered clean air Day 2: 1. activated carbon aerosol or 2. 1ppm SO_2 or 3. both Day 3: filtered clean air	4hr/day, 3days/wk for 3 consecutive wks.	10 male 10 female	20-35 yrs 22-33 yrs	20	Healthy	N/A	N/A

Observations:

Carbon had sorbed approximately 3-5% of its weight of SO_2 during the residence time in the chamber. Approximately two thirds of the sorbed SO_2 was converted to sulfate.
Mean decrements in function were greatest at t = 17min for all spirometric functions associated with the SO_2 and the combination (SO_2 +ACA) exposures. These changes were small, but statistically significant.

No statistically significant changes in spirometric function were observed with ACA exposure. No statistically significant changes in any pulmonary function

were observed 24 hr postexposure. No statistically significant differences resulted from the contrast between the SO_2 and combination exposures. Statistically significant increases occurred in nose or throat irritation with the SO_2 exposure in both eye and nose/throat irritation with the combination exposure;
*Statistically significant

Author's Conclusions:

The effect of the combination exposure is attributed to SO_2 alone.

The determination that the activated carbon sorbed only 1% of the SO_2 challenge concentration could explain the observed lack of SO_2 response enhancement.

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+/-)Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-) Are the subjects a representative sample of the population to which extrapolations will be made? (+) Was the method of recruitment appropriate? (Possibly – financial remuneration) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? (No info) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(+)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? (+)Were the chosen exposure levels appropriate to investigate the primary objective? (+/-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+/-)Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Were the subjects randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+)Is the control regime appropriate? (+)Was the type of blinding indicated? (*i.e.,* single, double, triple, etc)

	(+)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (+/-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+/-)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (N/A) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Carbon particles may play an important role as catalysts for many chemical reactions in the atmosphere. This study was designed to determine the effects of a low level (1.0 ppm) exposure to SO_2 and in combination with activated carbon aerosol. In this study, the effects of SO_2 exposure were not potentiated by the addition of the carbon aerosol. Statistically significant decreases in spirometric function were noted when the subjects were exposed to SO_2 and SO_2 + carbon, but not with carbon exposure alone. This lack of effects could also have been due to poor sorption by the carbon aerosol. The results in this study cannot be extrapolated to sensitive subpopulations. Significant decrements resulted following the first exercise associated with exposure initiation (t=17 min). The second exercise performed during exposure completion, however, produced little or no change in function (t=242 min).

Confidence index:

The subjects were put through an extensive screening process before being accepted to study. Selection bias may have been a problem. As indicated by the author, the exposures were randomized, but the sequence of days (control, exposure, postexposure) was not random. The activated carbon sorbed only 1% of the SO_2, which could have been why the response was not enhanced. There is reported consistency with other studies regarding exercise effects and possible attenuation in response to SO_2.

Review – Confidence index ranking:

High to Moderate

Author: Linn WS. Avol EL. Shamoo DA. Peng RC. Spier CE. Smith MN. Hackney JD.	**Internal ID: 097**

Title: Effect of metaproterenol sulfate on mild asthmatics' response to sulfur dioxide exposure and exercise.

Year: 1998

Abstract: Twenty asthmatic volunteers, most with mild disease, underwent dose-response studies with sulfur dioxide (SO_2) under three pretreatment conditions: (1) drug (metaproterenol sulfate in aerosolized saline solution), (2) placebo (aerosolized saline only), and (3) no pretreatment. Sulfur dioxide exposure concentrations were 0.0, 0.3, and 0.6 ppm. Experimental conditions were presented in random order at 1-wk intervals. Exposures lasted 10 min with heavy continuous exercise. Lung function was measured at baseline, after pretreatment (immediately pre-exposure), immediately post-exposure, and during a 2-hr follow-up. Subjects could elect to take bronchodilators during follow-up. Symptoms were monitored before, during, and for 1 wk after exposure. With no pretreatment, subjects exhibited typical exercise-induced bronchospasm at 0.0 ppm, slightly increased responses at 0.3 ppm, and more marked increases at 0.6 ppm. Seven subjects took bronchodilator after 0.6-ppm exposures, compared to 2 at lower concentrations. Within 30 min post-exposure, most subjects' symptoms and lung function had returned to near pre-exposure levels. A similar sequence was observed when subjects received placebo. Drug pretreatment improved lung function relative to baseline, prevented bronchoconstrictive responses at 0.0 and 0.3 ppm, and greatly mitigated responses at 0.6 ppm. Thus, typical bronchodilator usage by asthmatics is likely to reduce their response to ambient SO_2 pollution.

Objective: "This investigation was intended to quantitate the degree of protection conferred by a common adrenergic bronchodilator drug – metaproterenol sulfate – against physiologic and clinical effects of SO_2 exposures within a range which might be encountered in outdoor ambient air or in workplaces."

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0-0.6ppm	**10min**	**13M 7F**	**19-36**	**20**	**asthmatic**	**n/a**	**0.3ppm**

Observations:

With no pretreatment, subjects exhibited typical exercise-induced bronchospasm at 0.0 ppm, slightly increased responses at 0.3 ppm, and more marked increases at 0.6 ppm. Seven subjects took bronchodilator after 0.6-ppm exposures, compared to 2 at lower concentrations. Within 30 min post-exposure, most subjects' symptoms and lung function had returned to near pre-exposure levels. A similar sequence was observed when subjects received placebo.

*Statistically significant

Author's Conclusions:

"These results confirm previous qualitative reports that bronchodilator medications block the bronchoconstrictive effects of SO_2 and provide a quantitative estimate of the degree of protection that can be expected in typical mild asthmatics… In 0.3ppm exposures (encountered occasionally in ambient air near typical large SO_2 sources), metaproterenol pretreatment conferred nearly 100% protection against asthmatic responses. In 0.6ppm exposures (rare in ambient air, but more common in occupational settings), slight airway conctriction and symptoms occurred in at least a few individuals despite metaproterenol pretreatment, but no subject was affected enough to need more medication. Without drug pretreatment, the effects of SO_2 were sufficient to increase post-exposure medication usage noticeably at 0.6ppm, but not at 0.3ppm.

Review – Assessment:

A. Subjects:	+✏ Is the number of subjects sufficient to produce statistically significant results? +✏ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +✏ Are the subjects a representative sample of the population to which extrapolations will be made? +/-✏ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +/-✏ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✏ Was there a gradient of exposure levels? +✏ Were the chosen exposure levels appropriate to investigate the primary objective? -✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? +✏ Was evidence provided to justify the dosing regime? +✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	±✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined -✏ Was a full description of the monitoring devices provided? ±✏ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✏ Were the subjects randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✏ Was a control group assayed? +✏ Is the control regime appropriate? +✏ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✏ Was the length of follow-up appropriate? +✏ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical?

	-✓ Was raw data provided to permit the reader to arrive at his/her own conclusions? ±✓ Was individual data reported for each subject? ±✓ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✓ Are the statistical methods used appropriate? ±✓ Are confidence intervals reported? +✓ Did the investigator appropriately interpret the statistics? +✓ Are the observed trends statistically significant?
G. Interpretations:	+✓ Was the original objective addressed? +✓ Are the results novel? +✓ Was the design of the study appropriate to investigate the primary objective? +✓ Were assumptions reasonable? +✓ Are the methods reproducible? +✓ Did either the study design or the statistical analysis limit confounding variables? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the protective effect of metaproterenol sulfate on exposure to SO_2 with exercise in asthmatics. The results show that mild-asthmatics displayed bronchoconstriction even at 0 ppm SO_2 (EIB) that increased with SO_2 concentration. The concentrations used were environmentally relevant and could be encountered in ambient air. The pretreatment with metaproterenol gave nearly 100% protection to the asthmatic responses. The relevance of this paper is that mild asthmatics who exercise outdoors can use metaproterenol as a prophylactic treatment where there is a history of low level environmental pollution.

Confidence index:

The study design in this paper was logical and did not attempt to examine too many effects. Each subject was examined +/- drug treatment at three exposure levels (0, 0.3 and 0.6 ppm). The exposure levels were sufficient in obtaining an asthmatic response. By collecting lung function data at various time points shows the continued effect of both the drug and the SO_2. The pulmonary function data was adequately analyzed using ANOVA and further confirmed with subjective data obtained with a questionnaire. The results show that there is considerable inter-group variability, however, since each subject was exposed to all of the test exposures, comparison between groups is possible. The metaproterenol test group shows a marked protective effect that could not be attributable to other factors. The description of the exposure chamber and monitoring equipment was lacking however. No environmental variables (temp, humidity, chamber size, etc.) were reported so evaluation on the experimental design is not fully possible.

Review – Confidence index ranking:

Moderate to high

Author: McManus, M.S., Koenig, J.Q., Altman, L.C., Pierson, W.E. | **Internal ID:** 098

Title: Pulmonary effects of sulfur dioxide exposure and ipratropium bromide pretreatment in adults with nonallergic asthma

Year:1989

Abstract: In this study we examined the potential short-term effect of sulfur dioxide (SO_2) on total respiratory resistance and forced expiratory volume in patients with nonallergic asthma. A group of nine adult subjects with nonallergic asthma, 55 years of age or older, were exposed to SO2 at 0, 0.5, and 1.0 ppm for 20 minutes at rest followed by 10 minutes during light-moderate exercise. The measures of pulmonary function assessed were FEV_1, specific total respiratory resistance (SRT), and maximal expiratory flow rates at 50% (V_{max50}) and 75% (V_{max75}) of expired vital capacity. Measurements were made before exposure to SO_2 (baseline), postresting exposure, postexercising exposure, and at 30 minutes thereafter (recovery). Repeat measure analysis of variance revealed a statistically significant dose-response effect of SO_2 inhalation on FEV_1 ($p = 0.008$), SRT ($p = 0.033$), Vmax50 ($p = 0.017$), and V_{max75} ($p = 0.048$). Eight subjects had repeat exposure to SO_2 at 1.0 ppm after treatment with placebo or ipratropium bromide, 60 micrograms by metered-dose inhaler. ipratropium bromide treatment, compared to placebo treatment, resulted in a statistically significant improvement in all baseline measures of pulmonary function: FEV_1 ($p = 0.017$), SRT ($p = 0.027$), Vmax50 ($p = 0.018$), and V_{max75} ($p = 0.035$). However, this drug did not significantly alter the proportionate change in pulmonary function caused by SO_2 inhalation in these subjects. These findings indicate that adults with nonallergic asthma are sensitive to short-term low-level SO_2 exposure and that treatment with 60 micrograms of ipratropium bromide causes significant bronchodilation but does not protect, completely, these patients from the effect of SO_2 inhalation.

Objective: To examine the effect of SO_2 inhalation on the pulmonary function of subjects with nonallergic asthma and to characterize better the population with asthma responsive to low-level SO_2 inhalation. To examine the role of the muscarinic receptor in mediating this response.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Study 1: 1. filtered air 2. filtered air + 0.5ppm or 1.0 ppm SO_2	20 minutes at rest 10 minutes/light moderate exercise (10 min break between 20min and 10min exposures)	5 male 4 female	57-76 yrs	9	Asthmatic	N/A	N/A
Study 2: 1ppm SO_2 and ipratropium bromide	same as above			8			

Observations:

As a group, the subjects exhibited significant and consistent decreases in SR_T (specific total respiratory resistance) (p=0.033), FEV_1 (p=0.008), V_{max50} (p=0.017), and V_{max75} (p=0.048) after SO_2 exposure.

There was no significant increase in baseline pulmonary function values measured 10 minutes after medication administration with ipratropium bromide. However, 30 minutes after treatment with ipratropium bromide, pulmonary function measures improved significantly in spite of exposure to 1.0 ppm of SO_2 at rest. Ipratropium, bromide pretreatment did not alter the proportionate change in pulmonary function after exposure to SO_2 during light-moderate exercise.

*Statistically significant

Author's Conclusions:

Adults with nonallergic asthma have been found to respond in a dose-response fashion to short-term low-level SO_2 exposure. We have demonstrated that 60µg of ipratropium bromide results in a significant improvement in pulmonary function in these patients but does not attenuate the proportionate response to subsequent SO_2 inhalation. It appears that ipratropium bromide may provide some therapeutic benefit to subjects with nonallergic asthma caused by its ability to produce bronchodilatation by inhibiting baseline parasympathetic tone but may not protect these subjects from the effects of nonspecific (nonallergic) stimuli such as SO_2.

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-)Are the subjects a representative sample of the population to which extrapolations will be made? (+) Was the method of recruitment appropriate? (No info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+)Were subjects informed about the experimental risks? (Yes) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? (+)Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+/-)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+/-)Was evidence provided to justify the dosing regime?

	(+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+/-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+/-)Was a full description of the monitoring devices provided? (-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Were the subjects randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+)Was a control group assayed? (+) Is the control regime appropriate? (+)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+/-)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (+/-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+/-)Are the methods reproducible? (+/-)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (N/A)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study had two exposure concentrations and documented the effects of SO_2 on asthmatics. There appeared to be a statistically significant dose response effect of SO_2 exposure when the data was analyzed as a group. However, the magnitudes of responses largely varied among the subjects. Therefore, even at short-term low-level exposures, some subjects with nonallergic asthma may experience large deficits in pulmonary function. The pretreatment with ipratropium bromide resulted in bronchodilatation however, this drug did not block the proportionate change in pulmonary function with SO_2 inhalation . Different doses of the drug were not tried. The dose of 60 µg did not significantly inhibit the mechanism of SO_2

Induced bronchospasm in these subjects. This study provides evidence for human exposure guidelines for sensitive populations, and the data provided gives mean dose response curves from which to extrapolate physiologic reactions.

Confidence index:

This study used a very stringent selection protocol to screen for non-allergic individuals with asthma >55 yrs. As a result, a small test pool was available (n=9) but confounders were limited. The protocol for exposure was logical and was necessary due to the small test group. Each subject was exposed to all test conditions due to the potential for large individual variability. Experimental conditions were not detailed in the text and the exposure equipment was completely absent except for a reference. The data was adequately analyzed statistically, however, levels of significance in the tables would have aided in relating the data with the text material. Lack of information as to subject recruitment, follow up, withdrawals, source of reagents and sensitivity of monitoring equipment detract from this study.

The subjects were recruited from three large allergy clinics by chart review and telephone solicitation. The subjects also underwent an extensive selection process.

For the ipratropium bromide experiment, only eight of the subjects participated. There is no discussion on why the 9th subject was excluded.
There was large variation among subjects in the magnitude of response.

The authors report that the mean pulmonary function changes caused by SO_2 exposure were consistent and dose dependent. However, some of the standard errors of mean were quite large. For example, for the mean decreases in FEV_1 measured postresting exposure were 1.6%±0.8 with air exposure, 4.6%±1.9 with exposure with 0.05ppm of SO_2 and 8.5%±5.4 with exposure to 1.0 ppm of SO_2.

Review – Confidence index ranking:

Moderate

Author: Koenig JQ. Morgan MS. Horike M. Pierson WE	**Internal ID: 099**

Title: The effects of sulfur oxides on nasal and lung function in adolescents with extrinsic asthma.

Year: 1985

Abstract: Ten adolescent subjects with extrinsic asthma were exposed during intermittent exercise to filtered air, 0.5 ppm of sulfur dioxide (SO_2), or 100 $\mu gs/m^3$ of sulfuric acid (H_2SO_4) on 5 separate days. The purpose of the study was to compare changes in nasal power (the work of nose breathing) with pulmonary functional changes depending on the route of inhalation of the sulfur oxide pollutants, oral inhalation through a rubber mouthpiece or oronasal inhalation via a face mask. Nasal power was measured with a modified skin diving mask equipped with two differential pressure transducers. Statistically significant changes in total respiratory resistance, FEV_1, and maximum flow calculated at 50% and 75% vital capacity were observed after all exposures to SO_2 and H_2SO_4. The magnitude of change in FEV_1 and maximum flow calculated at 50% vital capacity was higher after oral compared to oronasal inhalation of SO_2. The nasal work of breathing increased 32% after SO_2 exposure by mouthpiece and 30% after SO_2 exposure via face mask (p less than 0.05). The nasal power changes after the H_2SO_4 exposures were not different from the sham exposures. We conclude that oronasal inhalation of 0.5 ppm of SO_2 produces a significant increase in the nasal work of breathing and that the route of exposure reduces but does not eliminate the lower airway reactions observed on oral exposure.

Objective: "The purpose of this experiment was to determine if bronchoconstrictor changes after SO_2 exposure were accompanied by parallel increases in nasal work of breathing when exposure was via a face mask. The experiment also was designed to compare nasal functional changes after SO2 exposure to those observed after H2SO4 exposure."

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.5ppm	**5 times - 50min**	**5M 5F**	**14-18**	**10**	**asthmatic**	**n/a**	**0.5ppm**

Observations:

"…the average increase in nasal power from baseline to 22min after exercise exposure was 30% after exposure to SO_2 through a face mask and 32% after exposure to SO_2 through a mouthpiece. There were no statistically significant changes comparing baseline to exposure No. 1 (10 min after exercise) values; however, the difference between baseline and after exposure No. 2 (22min after exercise) after SO_2 exposure via face mask was statistically significant both based on the t-test ($p<0.05$) and on the sign test ($p<0.008$). In general, there was an increase in the work of nose breathing after both exposures to SO_2 that was not observed after exposure to air or to H_2SO_4."

"There were statistically significant changes in the pulmonary functional parameters comparing baseline values to values after exercise exposure to either SO_2 or H_2SO_4…"

"The change from baseline to postexercise exposure after SO_2 exposure via mouthpiece was statistically different from the change after SO_2 exposure via facemask for FEV_1 and V_{max50}. In both cases the significance was $p<0.05$."

*Statistically significant

Author's Conclusions:

"We conclude that inhalation of 0.5ppm SO_2 via either route of entry (oral or oronasal) produced large changes in pulmonary function that were statistically significant from baseline values. We also conclude that oronasal inhalation of this concentration of SO_2 produces a significant increase in the nasal work of breathing and that this route of exposure reduces but does not eliminate the lower airway reactions observed on oral exposure."

Review – Assessment

A. Subjects:	+✏ Is the number of subjects sufficient to produce statistically significant results? +✏ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +✏ Are the subjects a representative sample of the population to which extrapolations will be made? ±✏ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +/-✏ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✏ Was there a gradient of exposure levels? +✏ Were the chosen exposure levels appropriate to investigate the primary objective? -✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✏ Were both nominal and actual exposure concentrations recorded? -✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? +✏ Was evidence provided to justify the dosing regime? +✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined ±✏ Was a full description of the monitoring devices provided? ±✏ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✏ Were the subjects randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✏ Was a control group assayed? +✏ Is the control regime appropriate? +✏ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✏ Was the length of follow-up appropriate? +✏ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical?

	-✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the impact of the route of exposure (mouth vs. nose) for SO_2 and H_2SO_4 exposure in adolescent asthmatics. The measurement of nasal work of breathing showed considerable variability within and between test subjects. Exposure to SO_2 increases the amount of nasal work with oronasal breathing. This increase in nasal work however does not totally reduce the lower airway reactions observed with oral exposure. The clinical significance of this study is that with normal breathing modes, SO_2 exposure causes more nasal breathing in asthmatics. This is especially important in exercise, where there are increased ventilation rates. The result is that the nasal passages may afford additional protection for the effects of SO_2 but does not totally negate the effects.

Confidence index:

As with most reports from this group, the experimental design is logical and appropriate to study the objectives. The number of subjects is small in this study due to very stringent selection procedures. All subjects were tested for allergies and had exercise induced bronchoconstriction. Limited information was given in the text about the experiment equipment and environmental variables and only a reference was given. However, previous papers by the same group show detailed information and confounders arising from the experimental setup are unlikely. The results showed marked effects, but the tables do not present the variability in the data in terms of the standard deviation. The data from a study such as this is expected to have large variability, however, the absence of such analysis makes the data interpretation difficult. Unfortunately, followup measurements after exposure were done for only a brief period of time, and may have missed recording clinically significant effects. Still, this is not expected to seriously affect the conclusions of the study.

Review – Confidence index ranking:

Moderate to high

Author: Magnussen, H., Jorres, R., Wagner, H.M., v.Nieding, G. **Internal ID:** 100

Title: Relationship between the airway response to inhaled sulfur dioxide, isocapnic hyperventilation, and histamine in asthmatic subjects

Year: ***1990***

Abstract: To determine whether bronchoconstriction induced by sulfur dioxide can be predicted by the airway response to inhaled histamine, we exposed on two days 46 patients with asthma to air or 0.5 ppm SO_2. The exposure protocol consisted of 10 min of tidal breathing followed by 10 min of isocapnic hyperventilation at a rate of 30 l/min. Airway response was measured before (baseline) and after hyperventilation in terms of specific airway resistance, SRaw. Exposure to air increased baseline mean (SD) SRaw from 6.27 (2.12) to mean (SD) maximum post-hyperventilation SRaw of 9.10 (4.38) cmH_2O*s (P less than 0.0001). Exposure to SO_2 increased mean (SD) baseline SRaw from 6.93 (3.29) to mean (SD) maximum post-hyperventilation SRaw of 18.21 (18.69) cmH2O*s (P less than 0.0001). Mean (SD) effect of SO_2 defined as difference between maximum post-hyperventilation SRaw after SO_2 versus air was 9.11 (16.14) cm H_2O*s. When evaluated individually, 26 and 34 of the 46 patients showed an airway response to hyperventilation of air and SO_2, respectively. Airway response to histamine was determined as the histamine concentration necessary to increase specific airway resistance by 100%, PC_{100}SRaw. The airway response after SO_2 and PC_{100}SRaw showed a weak but significant correlation (R = -0.48), whereas the responses to hyperventilation and SO_2 did not correlate. We suggest that the mechanisms by which histamine and SO_2 exert their bronchomotor effects are different and that in asthmatic patients the risk of pollutant-induced asthmatic symptoms can be poorly predicted by histamine responsiveness.

Objective: To determine whether the airway response to histamine and hyperventilation may be useful to predict the occurrence and magnitude of bronchoconstriction after exposure to SO_2.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.5ppm SO_2	20 minutes Consisting of: 10 minutes tidal breathing 10 minutes hyperventilation	25 women 21 men	16-62 years Control group: 24±5 yrs	46 Control group: 11	Asthmatic Healthy	N/A	N/A

Observations:

There were no statistically different findings in the control group for exposure to air or SO_2.

Airway response in the asthma group

Exposure to Air:

The increase in mean SRaw between post-hyperventilation and baseline was significant ($p<0.0001$).

Exposure to SO_2:

The increase in mean SRaw between post-hyperventilation and baseline was significant ($p<0.0001$).

Comparison of exposure to air and SO_2

The mean maximum post-hyperventilation SRaw after air and after SO_2 differed significantly ($p<0.0004$).
In 26 of 46 asthmatics, there was a significant airway response to hyperventilation of air, and in 34 of the 46 asthmatics, there was a significant airway response to hyperventilation of SO_2.

Histamine inhalation challenge

In the asthmatic subjects the geometric mean of PC_{100}SRaw as 0.88±3.68 mg/ml. The range was 0.045-9.75mg/mL. (only 1 patient was above 8mg/mL).

Comparison between airway response to air, SO_2, and histamine.

The maximum increase of SRaw after hyperventilation of SO_2 and the provocative concentrations PC_{100}SRaw of histamine showed a weak but statistically significant correlation ($r=-0.48$, $P=0.0004$).

*Statistically significant

Author's Conclusions:

We suggest that the mechanisms by which histamine and SO_2 exert their bronchomotor effects are different and that in asthmatic patients the risk of pollutant-induced asthmatic symptoms can be poorly predicted by histamine responsiveness.

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+/-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-)Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? (No info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+)Were subjects informed about the experimental risks? (No info) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? (+)Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined?

	(+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+)Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Were the subjects randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+)Was a control group assayed? (+) Is the control regime appropriate? (+)Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+/-) Was the length of follow-up appropriate? (+/-)Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
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F. Data analysis:	(+)Are the statistical methods used appropriate? (-)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-) Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense?

	(N/A)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study was designed to compare histamine and SO_2 response in the airways of asthmatic in order to determine if asthmatics sensitive to SO_2 could be determined using a histamine test. The findings in this study imply that in an individual asthmatic patient histamine-induced bronchoconstriction is only a poor predictor of the occurrence or magnitude of the airway response to SO_2. The findings also indicate that patients with bronchial asthma are more sensitive to inhaled SO_2 than normal subjects and that at low concentrations of SO_2 in almost all subjects an increased ventilation rate is necessary to elicit bronchoconstriction. The clinical relevance of this paper is that commonly used predictors of airway response are poor predictors of individual risk to general irritants such as SO_2.

Confidence index:

The strength in this study lies in the large number of subjects that were participating. Individual data given on the 46 asthmatics show a large variability in the subjects. This variability was reflected in the data and gives rise to many confounding variables such as medical treatment, smoking, age and general body mass. However, the experimental design was such that individual comparisons could be made as opposed to group comparisons. Experiment method was well described and most environmental conditions were reported. The data presentation in the text was cumbersome; the large subject pool made data interpretation difficult in tabular form. A graphical presentation would have made the data more convincing.

There was limited information provided on the control group. There were only 12 controls and the age ranges was 19-29yrs and all were nonsmokers. There was no mention of gender, and the results for control responses were not provided.

SRaw after hyperventilation of air and SRaw after hyperventilation of SO_2 were subtracted from each other and the difference referred to as effect of SO_2. However, the two exposures were performed on different days. This is not a reasonable assumption.

Review – Confidence index ranking:

Moderate to high

Author: Linn WS. Shamoo DA. Spier CE. Valencia LM. Anzar UT. Venet TG. Avol EL. Hackney JD.	**Internal ID: 101**
Title: Controlled exposure of volunteers with chronic obstructive pulmonary disease to sulfur dioxide.	
Year: 1985	
Abstract: Twenty-four volunteers with chronic obstructive pulmonary disease (COPD) were exposed to sulfur dioxide at 0, 0.4, and 0.8 ppm in a controlled environment chamber. Exposure lasted 1 hr and included two 15-min exercise periods, during which the mean ventilation rate was roughly 18 L/min. Pulmonary mechanical function was evaluated pre-exposure, after initial exercise, and at the end of exposure. Blood oxygenation was measured by ear oximetry pre-exposure and during the second exposure period. Symptoms were recorded throughout exposure periods and for 1 week afterward. No statistically significant changes in physiology or symptoms could be attributed to sulfur dioxide exposure. Older adults with SOPD seem less reactive to a given concentration of SO_2 than heavily exercising young adult asthmatics. This may be due to lower ventilation rates (i.e. lower SO_2 dose rates) and/or to lower airway reactivity in the COPD group.	
Objective: "This report describes a study intended to address the question (whether COPD enhances vulnerability to SO_2) more definitively."	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.0-0.8ppm	**1hr**	**15M 9F**	**49-68**	**24**	**COPD**		

Observations:

"Because there were no meaningful changes in group means, the physiologic data were reviewed to look for any atypical subjects who appeared to show increasingly unfavorable function changes with increasing SO_2 concentration. Only one such individual was found. His S_{aO2} (arterial oxygen saturation) data were consistent with the possibility that exercise in SO_2 exacerbated his arterial desaturation in a dose-related manner. He therefore underwent repeat exposure studies. They showed no desaturation with SO_2 exposure, suggesting that the original observation was due to chance."

In terms of symptoms: "The preexposure scores showed no significant variation among SO_2 levels except for nonrespiratory symptoms, and for them the significant difference was minimal…"

*Statistically significant

Author's Conclusions:

"No convincing evidence for untoward physiological or clinical effects of SO_2 exposure, for up to 1hr at concentrations of up to 0.8ppm, has been found in this subject population with COPD."

Review – Assessment:

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?

	+✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? +/-✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? -✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? -✎ Are the observed trends statistically significant?

G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings demonstrate that persons affected by COPD do not demonstrate deleterious effects in response to low-level SO_2 exposures. The authors mention that a potential explanation for the lack of significant results could be that these persons do not exercise as vigorously as other asthmatic or healthy persons, and may lower the risk of seeing SO_2 effects for that reason.

Confidence index:

The selection of subjects for this study was not appropriate to the objective. Although the number of subjects was significant (24), there was much variation within the group. The most important variable that could not be corrected for was the regular use of bronchodilators and steroids by some of the subjects. The use of these medications would have certainly imparted a protective effect manifesting itself during the studies. This could be reason why no effect was found for SO_2 exposure in the test group. The data analysis appeared to be performed adequately, however, no variability data (standard deviation for example) was given for the reader to evaluate. I suspect that there was much variability in the data stemming from the variability in the test group. This would have also made the data less significant and may have given a false negative result.

Review – Confidence index ranking:

Moderate

Author: Koenig, J.Q., Marshall, S.G., van Belle, G., McManus, M.S., Bierman, W., Shapiro, G.G., Furukawa, C.T., Pierson, W.E.	**Internal ID:** 102

Title: Therapeutic range cromolyn dose-response inhibition and complete obliteration of SO_2 induced bronchoconstriction in atopic adolescents

Year: 1990

Abstract: Eight atopic adolescent subjects with exercise-induced bronchospasm were studied to determine whether cromolyn sodium could inhibit or block sulfur dioxide (SO_2)-induced bronchoconstriction. Cromolyn or placebo was administered by turboinhaler 20 minutes before 10 minutes of SO_2 exposure at 1.0 ppm during continuous moderate exercise on a treadmill. The exercise level that was chosen did not in itself produce bronchoconstriction. The cromolyn doses were 0 (placebo), 20, 40, and 60 mg. Pulmonary functions (FEV1, and total respiratory resistance) were measured before and after drug administration and after exposure. SO_2 exposure after placebo produced significant bronchoconstriction. Pretreatment with 20 mg of cromolyn did not change the SO_2 response, 40 mg significantly inhibited the response, and 60 mg completely abolished the pulmonary function changes. These results demonstrate for the first time a dose-response inhibition of SO_2-induced bronchoconstriction in atopic subjects within a clinically acceptable dosage range and complete obliteration of SO_2 sensitivity in this group with 60 mg of cromolyn pretreatment

Objective: To determine whether cromolyn sodium could inhibit or block sulfur dioxide-induced bronchoconstriction in atopic adolescent subjects with exercise-induced bronchospasm.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
1. Placebo and 1.0 ppm SO_2 2. Cromolyn (20,40,or 60mg) and 1ppm SO_2	10 minutes exercise	6 female 2 male	13-17 yrs	8	atopic	N/A	N/A

Observations:

After placebo exposure during exercise, 1 ppm of SO_2 produced an average 14% decrease in FEV_1 ($p<0.025$) and an average 27% increase in R_T ($p<0.035$). Analysis of covariance with subjects as factors and dose as covariates with R_T values demonstrated a statistically significant treatment effect by cromolyn dose ($p<0.02$).

There were no statistically significant changes in functional residual capacity values or in symptom rating scores.

*Statistically significant

Author's Conclusions:

These results demonstrate for the first time a dose-response inhibition of SO_2-induced bronchoconstriction in atopic subjects within a clinically acceptable dosage range and complete obliteration of SO_2 sensitivity in this group with 60 mg of cromolyn pretreatment.

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+)Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? (No info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+)Were subjects informed about the experimental risks? (Yes) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? (+)Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+/-)Were the subjects randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+)Was a control group assayed? (+) Is the control regime appropriate? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams?

	(+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (+/-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (+)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (+/-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This paper investigates the dose relationship of cromolyn on the inhibition of SO_2 induced effects in atopics. The study demonstrated no inhibition of the SO_2 response with 20mg of cromolyn pretreatment. With 40 mg of cromolyn, the average decrease in FEV_1 was reduced from 14% after placebo to 7%, and the average increase in R_T was reduced from 27% after placebo to 1%. After 60mg of cromolyn, both SO_2 induced changes in FEV_1 and R_T were abolished.

Confidence index:

Lack of information as to method of recruitment, randomization, source and purity of reagents, sensitivity of monitoring equipment detract from this study. Furthermore, it is questionable why the investigators chose to test atopics rather than asthmatics in this study. Asthmatics would more likely benefit from the drug and would be more sensitive to SO_2. Their reasoning is that atopics do not required medication that could confound the study, however, mild-asthmatics can usually refrain from using medications before the study period. It is not surprising that the atopic group fully benefited from the administration of cromolyn. The authors conclusions stem from comparison with other studies, most involving asthmatics. These comparisons are not reasonable, as asthmatics have been previously shown to impart a different mechanism of bronchoconstriction compared to normals and atopics. They also conclude that cromolyn imparts multiple pharmacologic effects – this is not proven with the experimental design of the study. On the whole, the study does conclusively prove that cromolyn is useful for protection of SO_2 induced bronchoconstriction in atopics, however, extrapolation to other sections of the population is not reasonable.

Review – Confidence index ranking:

Moderate

Author: KOENIG JQ, MARSHALL SG, HORIKE M, SHAPIRO GG, FURUKAWA CT, BIERMAN CW, PIERSON WE	**Internal ID: 103**

Title: THE EFFECTS OF ALBUTEROL ON SULFUR DIOXIDE-INDUCED BRONCHOCONSTRICTION IN ALLERGIC ADOLESCENTS

Year: 1987

Abstract: Ten allergic subjects with exercise-induced bronchospasm were studied to determine whether albuterol could prevent sulfur dioxide (SO_2)-induced bronchoconstriction. Albuterol or placebo (180 micrograms) were administered by metered-dose inhaler 20 minutes before a 10-minute exposure to SO_2 or clean air during moderate exercise on a treadmill at an exercise level that by itself did not produce exercise-induced bronchospasm. Pulmonary functions (FEV1 and total respiratory resistance [RT]) were measured before the drug, after the drug, and after exposure to SO_2 or clean air. Albuterol treatment produced significant bronchodilation and also prevented SO_2-induced bronchoconstriction. Following SO_2 inhalation after placebo, FEV1 decreased 15% (p less than 0.02) and RT increased 50% (p less than 0.03). Following SO_2 inhalation after albuterol treatment, neither FEV1 nor RT changed significantly. We conclude that albuterol, a beta 2-agonist, inhibits SO_2-induced bronchoconstriction. This result suggests that the adrenergic nervous system or mast cell degranulation are involved in SO_2-induced bronchoconstriction.

Objective: To determine if albuterol can prevent SO2 induced bronchoconstriction in allergic adolescents with exercise induced bronchospasm.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.75ppm	**10 min**	**7 female** **3 male**	**13-17**	**10**	**Allergic without extrinsic asthma**	n/a	n/a

Observations:

Ten allergic subjects with exercise-induced bronchospasm were studied to determine whether albuterol could prevent sulfur dioxide (SO_2)-induced bronchoconstriction. Albuterol or placebo (180 µg) were administered by metered-dose inhaler 20 minutes before a 10-minute exposure to SO_2 or clean air during moderate exercise on a treadmill at an exercise level that by itself did not produce exercise-induced bronchospasm. Pulmonary functions (FEV_1 and total respiratory resistance [R_T] were measured before the drug, after the drug, and after exposure to SO_2 –induced bronchoconstriction. Following SO_2 inhalation after placebo, FEV_1 decreased 15% ($p<0.02$) and R_T changed significantly.

*Statistically significant

Author's Conclusions:

Two main conclusions are noted:

1) Albuterol produced bronchodilation as demonstrated by the postdrug baseline pulmonary function measurements.
2) Albuterol prevented SO_2-induced bronchoconstriction in test subjects.

Review – Assessment:

A. Subjects:	-✏ Is the number of subjects sufficient to produce statistically significant results? +✏ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? -✏ Are the subjects a representative sample of the population to which extrapolations will be made? ±✏ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +/-✏ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✏ Was there a gradient of exposure levels? +✏ Were the chosen exposure levels appropriate to investigate the primary objective? ±✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? +✏ Was evidence provided to justify the dosing regime? +✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✏ Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✏ Were the subjects randomly assigned to exposure groups? +✏ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? -✏ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✏ Was the length of follow-up appropriate? +✏ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✏ Were accepted assessment methods employed?

	+✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? -✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? ±✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? -✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings of this study appear to be legitimate, in that albuterol does apparently reduce bronchoconstriction in SO_2 exposed individuals. However, this study does not investigate what the human health effects of SO_2 exposure are, instead it explicitly uses SO_2 exposure to trigger bronchoconstriction. SO_2 exposure for 10minutes at 0.75ppm does induce bronchoconstriction in this study. Suggestion that the adrenergic nervous system or mast cell degranulation are involved point the way to further study that would isolate the mechanisms of SO_2 induced bronchoconstriction, and more effective medication.

Confidence index:

The study design does appear to be valid, as well as the results obtained. However, they do not directly apply to the topic of interest (health effects of SO_2 exposure). The study is also supported by Glaxo Laboratories, a drug company with a vested interest in seeing significant results. While the study investigated a range of confounding factors for the subjects, history of smoking was not investigated. In addition, the number of subjects included in the investigation (10) was not enough to produce statistically relevant results. Limited mention was made of how subjects were recruited to the study, and whether incentive was employed was not mentioned. In addition, it was not recorded whether subjects were made aware of the risks associated with the studies. Finally, no rationalization was given for selection of the exposure level of SO_2.

Review – Confidence index ranking:

Moderate to high

Author: Melville GN	**Internal ID: 105**
Title: Changes in specific airway conductance in healthy volunteers following nasal and oral inhalation of SO_2.	
Year: 1970	

Abstract: Abstract: In modern industrial society man is exposed to an increasing number of atmospheric pollutants. One of these pollutants is sulphur dioxide, which in low concentrations affect the upper respiratory tract resulting in increases in airway resistance. Anderson however reported that human beings continually exposed to SO_2 experienced no subjective discomfort and Goldring et al found minimal pathological changes in hamsters exposed to So2 for 75 days. Recently, Speizer et al , using the esophageal catheter technique, showed that changes in pulmonary flow resistance were related to the pathway of SO_2 entry into the respiratory system.

Differences in response of the tracheobronchial tree to SO_2 might be expected if the nose and mouth were unequal in their ability to absorb SO_2 or if receptior tissue capable of initiating bronchoconstriction was more abundant in either of the two pathways. This paper reports studies done using a body plethysmograph, to assess,

a) specific airway conductance changes following inhalation of various concentrations of SO_2 by nose and mouth for periods ranging from 1-60 minutes and

the relative clearances of SO_2 during nose and mouth breathing

Objective: "This paper reports studies done using a body plethysmograph, to assess:

a) specific airway conductance changes following inhalation of various concentrationfs of SO_2 by nose and mouth for periods ranging from 1-60minutes, and

b) the relative clearances of SO_2 during mouth and nose breathing.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
2.5-10ppm	**10min-1hr**	**n/a**	**n/a**	**49**	**healthy**	**n/a**	**2.5ppm**

Observations:

"sGaw (specific airway conductance) decreased significantly ($p<0.05$) in 75% of the subjects studied when SO_2 was inhaled by nose. With mouth breathing, sGaw decreased significantly ($p<0.05$) in 80% of the subjects. The percentage decrease was greater with mouth breathing than with nose breathing at all concentrations. The greatest percentage change in sGaw, irrespective of the path of entry, was seen after 5min exposure to SO_2 at all concentrations."

"…the results show that more SO_2 was cleared per minute with notes breathing than with mouth breathing."

*Statistically significant

Author's Conclusions:

"Specific airway conductance (sGaw) was measured using a body plethysmograph in 49 healthy volunteers exposed by nose and mouth to sulfur dioxide (SO_2) below and above the maximum daily allowable concentration of 5ppm for periods of up to 1hour. SO_2 caused a decrease in sGaw with both nose and mouth breathing at low concentrations. The decrease in sGaw was greater with mouth breathing. At high concentration (above 5ppm) however, there was no significant difference between nose and mouth breathing. In subjects exposed to 5ppm SO_2 for up to 1 hour, there was no further significant decrease in sGaw after 5min. These results suggest that changes in sGaw following SO_2 inhalation are related to stimulation of tracheobronchial receptors and that after a period acclimatization occurs."

Review – Assessment

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? -✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ±✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? ±✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? -✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? +/-✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) ±✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? -✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom)

	+✓ Were accepted assessment methods employed? +✓ Were assessment measures sufficiently sensitive? +✓ Was the nature and severity of symptoms graded against an explicitly defined scale? +✓ Did qualified personnel conduct the exams? +✓ Was the timing and frequency of assessment logical? -✓ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✓ Was individual data reported for each subject? ±✓ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✓ Are the statistical methods used appropriate? ±✓ Are confidence intervals reported? +✓ Did the investigator appropriately interpret the statistics? +✓ Are the observed trends statistically significant?
G. Interpretations:	+✓ Was the original objective addressed? +✓ Are the results novel? +✓ Was the design of the study appropriate to investigate the primary objective? +✓ Were assumptions reasonable? +✓ Are the methods reproducible? -✓ Did either the study design or the statistical analysis limit confounding variables? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This was an older study investigating the significance of the route of exposure on the effect of SO_2. The results show that the mouth is less effective than the nose in removing SO_2 from inspired air. Continued exposure to SO_2 resulted in no significant change in airway conductance (resistance). The clinical relevance of this paper is that at SO_2 concentrations at 5 ppm and above, normal individuals are likely to experience bronchoconstriction which is attenuated with nose breathing.

Confidence index:

The methods section of this paper is quite poor and vague in its description of both the study subjects and the research methods. The subject characteristics were not described at all in terms of age, sex, or employment, nor were the methods by which they were recruited, meaning the study subjects might not be representative of the overall population, affecting the generalizability of results. Furthermore, no mention was made of the types of statistical analyses used.

The experimental setup was crude, but adequate to deliver the SO_2 and monitor the airway conductance reasonably. Additional information about the exposure conditions such as temperature and humidity would have aided interpretation. The study design was simplistic as all of the subjects were exposed to all of the test conditions. Simple screening of the subjects would have increased the significance of the results and decreased the variability of the data.

Review – Confidence index ranking:

Moderate to low

Author: Harries, M.G., Parkes, P.E.G., Lessof, M.H.	**Internal ID:** 108
Title: Role of Bronchial Irritant Receptors in Asthma	
Year: 1981	
Abstract Sulphur dioxide inhaled at low concentration had no detectable effect on forced expiratory volume in one second in non-asthmatic atopic controls, but it induced asthma and increased bronchial reactivity in nine symptomless atopic and non-atopic asthmatic patients. This increased reactivity was inhibited by sodium cromoglycate (SCG) and partly inhibited by atropine. This suggests that SCG, in addition to its ability to stabilize mast cells, may also act on bronchial irritant receptors or directly on smooth muscle in asthmatic patients.	
Objective: We produced a reproducible stimulus to bronchoconstriction on repeated occasions with a consistency greater than that provided by bronchial provocation with soluble antigens.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
5-11.5 ppm	No clear information on duration	No info	15-55 yrs	9	Asthmatic	N/A	N/A
15 ppm				6	Non-asthmatic,atopic		
10 min before SO_2 exposure, sodium cromoglycate (40mg), placebo, or 0.5% atropine methonitrate were taken							

Observations:

It is not indicated whether any of the results are statistically significant. In the non-asthmatic patients the FEV_1 decreased by approx. .1±0.27 litres after exposure to 15ppm SO_2. In the asthmatic patients breathing SO_2 at concentrations 5-11.5 ppm, the FEV_1 also decreased 1.3 litres±0.2. Pre-treatment with 40 mg sodium cromoglycate resulted in a fall in FEV_1 of 0.3 litres±0.23 and after 0.5% atropine sulphate given by aerosol FEV_1 fell by 0.2 litres±0.25.
*Statistically significant

Author's Conclusions:

The results suggest, that sodium cromoglycate, in addition to its ability to stabilize mast cells, may also act on bronchial irritant receptors or directly on smooth muscle in asthmatic patients.

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (-) Was the method of recruitment appropriate? (No info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (-) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? (-)Were the chosen exposure levels appropriate to investigate the primary objective? (+)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (-)Was the duration of exposure appropriate to investigate the primary objective? (-)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+/-)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Were the subjects randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+)Was a control group assayed? (+) Is the control regime appropriate? (-)Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+) Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom)

	(+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+/-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (N/A)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-)Are the statistical methods used appropriate? (-)Are confidence intervals reported? (N/A)Did the investigator appropriately interpret the statistics? (N/A)Are the observed trends statistically significant?
G. Interpretations:	(-)Was the original objective addressed? (-)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (+)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables? (-)Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the inhibitory effect of cromoglycate (SCG) on bronchoconstriction induced by SO_2 in asthmatics. The results showed that asthmatics required a smaller dose of SO_2 to produce a fall in FEV of 20% compared to a group of normal subjects. Pretreatment with 40 mg of SCG was shown to reduce the bronchoconstriction compared to the placebo more than pretreatment with 0.5% atropine. Neither treatment was able to fully block the effects of the SO_2 dose. There were no statistically significant findings in this study.

Confidence index:

The subject pool size was marginal (n=15), however only 9 were used for direct study while the other 6 were used only as a comparison for SO_2 sensitivity. Almost no information about the subjects was given including medical history, work and environmental exposures, ht, wt, smoking, etc. In addition, the objective was not clearly stated and the experimental setup was not detailed in the text. No indication on the route of exposure (mouth or nose) was given along with temperature and humidity. Comparison of the two drugs was insufficient; a dose-response comparison is needed to fully ensure that the observed effects are true and not due to under/over medication. There was no statistical analysis except for reporting of the standard deviation. Significance testing was not performed although the data was available.

Review – Confidence index ranking:

Low

Author: Jorres R. Magnussen H. **Internal ID: 109**

Title: Airways response of asthmatics after a 30 min exposure, at resting ventilation, to 0.25 ppm NO_2 or 0.5 ppm SO_2.

Year: 1990

Abstract: We compared the effect of inhaled NO_2 and SO_2 on airway tone and airway responsiveness in 14 nonsmoking mild asthmatics (mean +/- SD age 34 +/- 14 yrs; mean +/- SD baseline forced expiratory volume in one second (FEV1), 86 +/- 17% pred). On 3 separate days, 30 min tidal breathing (average minute ventilation 10.6 l.min-1) of either filtered air, 0.25 ppm NO_2, or 0.5 ppm SO_2 was followed by an isocapnic hyperventilation test with 0.75 ppm SO_2. To determine the provocative ventilation necessary to increase specific airway resistance (SRaw) by 100% (PV100SRaw) ventilation was increased in steps of 15 l.min-1, each step lasting 3 min. Resting ventilation of filtered air, NO_2, or SO_2 was followed by a slight but significant overall decrease of SRaw from 8.8 to 7.7 cmH20.s-1, with no differences between the study days. Mean +/- SEM PV100SRaw(SO2) was 46.5 +/- 5.1, 37.7 +/- 3.5 and 45.4 +/- 4.2 l.min-1 after tidal breathing of filtered air, NO_2, and SO_2, respectively. PV100SRaw(SO_2) was significantly lower after NO_2 as compared to filtered air or SO_2 (p less than 0.01). There was a significant correlation (rs = 0.86) between the individual shift of PV100SRaw(SO_2) after NO_2 and the shift after SO_2 as compared to filtered air. From these individual comparisons we suggest that in asthmatics short-term exposure to NO_2 at rest enhances airway responsiveness to hyperventilation of SO2 without altering airway tone, whereas short-term exposure with low concentrations of SO_2 does not.

Objective: We compared the effect of inhaled NO_2 and SO_2 on airway tone and airway responsiveness in 14 nonsmoking mild asthmatics.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.5-0.75ppm	**30min tidal breathing then hyperventilation 15 min after**	**10M 4F**	**20-55**	**14**	**asthmatic**	**n/a**	**n/a**

Observations:

"Thirty min of tidal breathing of either filtered air, 0.25ppm NO_2, or 0.5ppm SO_2 induced a small but significant ($p<0.05$) overall mean decrease of SRaw from 8.8 to 7.7cmH20/ l s with no differences between study days."

"Mean±SEM provocative ventilation PV100SRaw (SO_2) necessary to increase SRaw during isocapnic hyperventilation of 0.75ppm SO_2 following tidal breathing of filtered air, 0.25ppm NO_2 or 0.5ppm SO_2 were 46.5±5.1, 37.7±3.5, and 45.4±4.2l/min, respectively. PV100SRaw(SO_2) after NO_2 was significantly lower ($p<0.01$) than both PV100SRaw(SO_2) after filtered air or 0.5ppm SO_2, which did not differ statistically from each other."

*Statistically significant

Author's Conclusions:

"Our observations demonstrate that tidal breathing of 0.25ppm NO_2 for 30min did not increase airway tone but enhanced airway responsiveness to subsequent hyperventilation of 0.75ppm SO_2. This is in contrast to the effect of tidal breathing of 0.5ppm SO_2 which neither caused airway obstruction nor changed airway responsiveness."

"The change of airway responsiveness to SO_2 hyperventilation after NO_2 exposure may lead to the speculation that inhaling NO_2 enhances airway responsiveness to other gaseous pollutants, e.g., SO_2. Though this reasoning is only based on indirect evidence, our observation may point to the possibility of interactions between the gaseous constituents of polluted air."

Review – Assessment:

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ±✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? +/-✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? +✎ Is the control regime appropriate? +✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale?

	+✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the airway response due to resting ventilation of 0.25 ppm NO_2 or 0.5ppm SO_2 + challenge with 0.75 ppm SO_2 with isocapnic hyperventilation. Results were given for both 0.25 NO_2 and 0.5 SO_2 but only the SO_2 result will be discussed. The results show that resting ventilation with 0.25 ppm SO_2 does not cause airway obstruction or airway responsiveness. This result is in line with other studies showing that bronchoconstriction is not apparent at 0.25 ppm SO_2 with tidal inhalation.

Confidence index:

This report consisted of a meticulous study, which was presented, in great detail in the text. The number of subjects is marginal for a significant study, however, the subjects were screened stringently and their clinical conditions were summarized in detail. The experimental setup gave a detailed account of the protocol, exposure equipment/conditions, and monitoring. The data was presented clearly in tabular format showing individual data, however, the levels of significance were shown only in the text. It is unclear however, why the authors chose to examine the pretreatment of subjects with tidal breathing instead of looking at gas interactions. Pretreatment of subjects with 0.25 ppm SO_2 makes no sense as this would be considered a short-term exposure. The same goes for the pretreatment with NO_2. While the data presented here is significant and valid, little valuable information resulted from this study.

Review – Confidence index ranking:

High to moderate

Author: Gunnison, A.F., Palmes, E.D. **Internal ID:** 112

Title:S-Sulfonates in Human Plasma following Inhalation of Sulfur Dioxide

Year: 1974

Abstract: Plasma *S*-sulfonate was measured in human subjects exposed continuously under controlled conditins to sulfur dioxide for periods up to 120 hours. Subjects were exposed to chamber SO_2 concentratios of 0.3, 1.0, 3.0, 4.2, and 6.0 ppm and to a control exposure with ambient air. Plasma levels of *S*-sulfonate showed positive correlation with atmospheric sulfur dioxide; the regression line fitted to all 80 data points determined in this experiment resulted in a highly significant correlation (correlation coefficient = 0.61, $p<0.001$). The slope of the regression line was an estimated increase of 1.1 ± 0.16(S.E.) nmoles of *S*-sulfonate per milliliter of plasma for each ppm increment in chamber sulfur dioxide concentration.

Objective: The purpose of the experiments reported here was to investigate the possible production of plasma *S*-sulfonate in humans as a function of controlled exposures to sulfur dioxide.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0,0.3,1.0,3.0,6.0ppm	120 continuous hours at each conc.	Male	Mean 22yrs	Group 1: 13	Healthy nonsmokers	N/A	N/A
0,0.3,1.0,3.0ppm	96 continuous hours at each conc.	male	Mean 34 yrs	Group 2: 7	Heavy smokers		

Observations:

Note on Above Study Design Data:
One subject in group 1 was exposed to 3.0 and 6.0 ppm of SO_2 for 48 continuous hours.
In addition, two other subjects in Group 1 reeived these two 48-hour exposures.
Two subjects of Group 2 were additionally exposed to 4.2 ppm of SO_2.

Observations:
Relationship between Atmospheric SO_2 and Plasma S-Sulfonate
The plasma levels of *S*-sulfonate showed positive correlation with atmospheric sulfur dioxide.
When the regression line was fitted to all 80 data points (51 from Group 1 and 29 from Group 2), the correlation was significant ($p<0.001$) at $r=0.61$ with an

estimated increase of 1.1±0.16 nmoles of plasma S-sulfonate per milliliter for each 1 ppm increment in chamber SO_2 concentration.
*Statistically significant

Author's Conclusions:

No clear conclusions were made.

Review – Assessment:

A. Subjects:	(-)Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? (No info provided) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+/-) Were subjects informed about the experimental risks? (N/A) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-)s the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+/-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+/-) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+)Is the control regime appropriate? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+/-)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)

E. Data collection:	(+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (-) Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (+/-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables? (N/A)Do the conclusions make biological sense? (N/A)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the relationship between S-sulfonates in human plasma and the dose of SO_2 exposed to adults. The results show that there is a linear relationship between the dose of SO_2 and the amount of S-sulfonate recovered in the plasma. The significance of this is a possible biomarker that is easily recovered for SO_2 exposure. The significance of S-sulfonate in human plasma is not discussed.

Confidence index:

There are many limitations of this study. The first involves the subjects. The number of subjects is sufficient, however, little information about their previous clinical history was given leading to possible confounders. Regardless, the investigators pooled the data between the heavy smokers and the non-smokers because regression results were not significantly different. This is not a valid assumption as the variance in the data may have caused the insignificance and not the actual response by the two groups. Lack of information as to reagent source, monitoring equipment, method of recruitment, follow up, method or randomization, method of administration all detract from this study. There is no information on the subjects besides their smoking status (non or heavy), gender and mean age.

Nor is there information on whether any of the subjects were exsmokers.

The recovery data indicated that the authors were working very close to the limit of sensitivity of the analytical method at the levels of S-sulfonate encountered in this experiment.

Review – Confidence index ranking:

Low

Author: Burton GG, Corn M, Gee JBL, Vasallo C, Thomas AP	**Internal ID: 113**
Title: Response of healthy men to inhaled low concentrations of gas-aerosol mixtures.	
Year: 1969	
Abstract: n/a	
Objective: To expand the results of previous studies by examining airway resistance (Ra) and pulmonary flow resistance (R_L) during "…precisely controlled and characterized pollutant exposures."	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1.1-3.6ppm	**30min**	**Male**	**25-34**	**10**	**healthy**	**~3ppm**	**n/a**

Observations:

"…no significant increases in C_L (compliance) and R_L (pulmonary flow resistance) were seen during quiet breathing or during hyperventilation, either after SO_2 alone, or after the SO_2-aerosol mixture. Similarly, no significant changes in R_A (airway resistance), airway conductance (G_A) or specific conductance could be demonstrated."
*Statistically significant

Author's Conclusions:

"This study confirms existing evidence that human exposures to low concentrations (<3.0ppm) of SO_2 in air do not result in immediate physiologic effects on measures of pulmonary mechanics." "In summary…we could not demonstrate gas-aerosol synergism for SO_2 and inert aerosols at concentrations which approximate those in urban atmospheres."

Review – Assessment:

A. Subjects:	+✏ Is the number of subjects sufficient to produce statistically significant results? ±✏ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ±✏ Are the subjects a representative sample of the population to which extrapolations will be made? ±✏ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +/-✏ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	+✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✏ Was there a gradient of exposure levels? +✏ Were the chosen exposure levels appropriate to investigate the primary objective?

	-✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? +/-✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	-✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? -✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? -✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective?

	+✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This was an older study involving the effect of a SO_2-aerosol mixture on "normal" male subjects. The results show that 2.0 ppm SO_2 alone or with a NaCl aerosol did not significantly change any endpoint of pulmonary function. These results confirmed other existing studies performed at the time and later on. This result is not surprising, as the subjects were not stressed by exercise. However, it does provide some support for the guidelines because the study tested low concentrations of SO_2 (2.0 ppm) on the subjects. It does provide some support for exposure of the general, robust population, or a ceiling level to which sensitive sub-populations should not go beyond.

Confidence index:

The number of subjects in this study is marginal to low (n=10) and involved both smokers and non-smokers. The subjects were also not stringently screened before the procedure. These factors would lead to confounders and since smokers and non-smokers were not separated for the study, large variation in the data would be expected and was observed. This variation caused the data analysis difficult and any small changes would not be detected with the experimental and statistical design. Also, the levels of exposure to SO_2 was variable due to the exposure device. The variation of SO_2 concentration between subjects ranged from 1.2-3.2 ppm which translates into some subjects receiving almost 2.5 times the concentration compared to others.

Review – Confidence index ranking:

Moderate

Author: Fine, J.M., Gordon, T., Sheppard | **Internal ID:** 116

Title: The Roles of pH and Ionic Species in Sulfur Dioxide-and Sulfite-Induced Bronchoconstriction

Year: 1987

Abstract: Sulfur dioxide (SO_2) and sulfites are well-described causes of bronchoconstriction in persons with asthma that are chemically related and, therefore, may share a common mechanism of action. When either sulfur species dissolves in aqueous solutions, a pH-dependent equilibrium is established predominantly among bisulfite ion (HSO3-), sulfite ion (SO^{3-}), and SO_2. In addition, hydrogen ions may be released. To assess the relative bronchoconstricting potencies of these chemical forms and the role of acidity caused by the release of hydrogen ions in SO_2. and sulfite-induced bronchoconstriction, we administered to 10 asthmatic subjects nebulized sodium sulfite (Na2SO3) solutions at pH 9 containing 95% sulfite, at pH 6.6 containing 80% bisulfite, and at pH 4 containing 99% bisulfite but greater than an order of magnitude more SO_2 than the pH 6.6 solutions. Subjects inhaled increasing concentrations of aerosolized Na_2SO_3 at each pH during 1 min of tidal breathing. Subjects also breathed buffered acetic acid aerosols with the same acidity of the pH 4 Na_2SO_3 solutions to control for the airway effects of acid aerosols. To assess sensitivity to SO_2 gas, subjects inhaled increasing concentrations of SO_2 during eucapneic hyperpnea. Bronchoconstrictor response was assessed by measuring specific airway resistance (SRaw) before and after each challenge. Nine of the 10 subjects developed bronchoconstriction after inhaling the Na_2SO_3 aerosols at all 3 levels of pH and the SO_2 gas. The mean concentration of Na_2SO_3 solution calculated to increase SRaw by 100% above baseline was significantly different (p less than 0.01) at the various levels of pH: pH 4 (0.17 mg/ml) less than pH 6.6 (0.49 mg/ml) less than pH 9 (2.10 mg/ml). Only 1 subject responded to the acetic acid aerosol. These results suggest that bronchoconstriction provoked by inhalation of sodium sulfite aerosols may be caused by the SO_2 gas released or by bisulfite ions, but not by sulfite ions. Furthermore, SO_2 and bisulfite are not likely to cause bronchoconstriction merely as a result of an alteration in airway pH.

Objective: The purpose of the present study was to determine the relative contributions of bisulfite, sulfite ion, SO_2, and change in airway pH to bronchoconstriction caused by inhalation of these sulfur species.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
1. Sodium sulfite at pH 9 95% sulfite 2. Sodium sulfite at pH 6.6 containing 80% bisulfite 3. Sodium	1 min of tidal breathing	5 male 5 female	22-34 yrs	10	asthmatic	N/A	N/A

sulfite at pH 4 99% sulfite (0.03,0.1,0.3,1.0, 3.0, and 10.0 mg/ml 4. SO_2 (0.25,0.5,1.0,2.0, 4.0,8.0 ppm)	1 minute during eucapneic hyperpnea						

Observations:

Nine of 10 subjects studied developed significant bronchoconstriction after inhalation of sulfite aerosols at all 3 levels of pH studied.

In each of the 9 subjects who developed sulfite-induced bronchoconstriction, a 100% increase in SRaw occurred at the lowest sulfite concentrations at pH 4.0 and at the highest concentration at pH 9.The mean PC_{100} values were significantly different at each pH. This effect occurred at a total (titrable) acidity more than 70 times higher than the acidity delivered in the concentration of sodium sulfite at pH 4 required to cause equivalent bronchoconstriction.

No sulfur dioxide gas was detectable during aerosolization of sodium sulfite at pH 9 except at the highest concentration (10mg/ml) where we measured 0.02 ppm SO_2. Sulfur dioxide gas was detectable during aerosolization of sodium sulfite at pH 4 and pH 6.6. Aerosols of pH 4.0 solutions yielded approximately 20 times more SO_2 than did those of pH 6.6 solutions at concentrations of 0.1, 0.3, and 1.0 mg/ml.

The relationship between SO_2 concentration and sulfite concentration was not linear, and the difference between the SO_2 concentrations at the 2 levels of pH was far less than that predicted by the equilibrium equations described above.

*Statistically significant

Author's Conclusions:

The results of this study confirm the reports of other investigators that inhaled sulfite aerosols are a stimulus to bronchoconstriction in subjects with asthma.

Although we cannot determine whether SO_2 or bisulfite is the more potent bronchoconstricting stimulus, biochemical and in vitro evidence points toward bisulfite.

Our findings that most patients with asthma develop bronchoconstriction after inhalation of sodium sulfite aerosols suggest that the practice of identifying patients thought to have "specific sulfite sensitivity" on the basis of a bronchoconstrictor response to inhaled sulfites is not valid, even if the specific conditions of aerosolization do not generate significant concentrations of SO_2. Second, our results suggest that sulfite aerosols, such as those formed in the atmosphere by the interaction of liquid water and SO_2, are capable of causing bronchoconstriction, especially when inhaled at acid pH.

Review – Assessment:

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results?

	(+/-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-)Are the subjects a representative sample of the population to which extrapolations will be made? (+) Was the method of recruitment appropriate? (No info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? (Yes) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (-)Was the duration of exposure appropriate to investigate the primary objective? (-)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+)Was a full description of the monitoring devices provided? (+) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+)Is the control regime appropriate? (+)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (+/-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive?

	(-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+/-)Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+)Was individual data reported for each subject? (+/-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (-)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+/-)Was the original objective addressed? (+/-)Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+/-)Are the methods reproducible? (+/-)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (N/A)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

10 asthmatic subjects, evenly split between male and female, were exposed to increasing concentrations of sulfur dioxide to establish a baseline of response from which to grade exposure reactions to other similar irritant gases. The findings of this study basically indicate that the bronchoconstriction provoked by inhalation of sodium sulfite aerosols may be caused by; the SO_2 gas released or by bisulfite ions, but not by sulfite ions. The findings also indicate that the hydrogen ions released are not likely to be the sole cause of bronchoconstriction.

Confidence index:

Minor deficiencies in information as to source of reagents, randomization, sensitivity of monitoring devices detract from complete confidence in this study. Furthermore, the sample size is small and the results of the SO_2 exposures are not included in the results section.

The SO_2 exposures were only 1 minute per concentration and then a 10-minute interval. There is no justification for the exposure duration or frequency. The effects at one concentration could be due to the previous exposure.

Review – Confidence index ranking:

Low

Author: Bethel, R.A., Sheppard, D., Geffroy, B., Tam, E., Nadel, J.A., Boushey, H.A.

Internal ID: 118

Title: Effect of 0.25ppm Sulfur Dioxide on Airway Resistance in Freely Breathing Heavily Exercising, Asthmatic Subjects

Year: 1985

Abstract: We sought to determine whether 0.25 ppm sulfur dioxide in filtered air causes bronchoconstriction when inhaled by freely breathing, heavily exercising, asthmatic subjects. Nineteen asthmatic volunteers exercised at 750 kilogram meters/min for 5 min in an exposure chamber that contained filtered air at ambient temperature and humidity or, on another day, filtered air plus 0.25 ppm sulfur dioxide. The order of exposure to sulfur dioxide and to filtered air alone was randomized, and the experiments were double-blinded. Specific airway resistance, measured by constant-volume, whole-body plethysmography, increased from 6.38 +/- 2.07 cm H2O X s (mean +/- SD) before exercise to 11.32 +/- 8.97 after exercise on days when subjects breathed filtered air alone and from 5.70 +/- 1.93 to 13.33 +/- 7.54 on days when subjects breathed 0.25 ppm sulfur dioxide in filtered air. The increase in specific airway resistance on days when subjects breathed 0.25 ppm sulfur dioxide was only slightly greater than on days when they breathed filtered air, but the difference was significant. To determine whether 0.25 ppm sulfur dioxide causes greater bronchoconstriction in asthmatic subjects exercising more vigorously, 9 subjects then repeated the experiment exercising at 1,000 instead of 750 kilogram meters/min. Specific airway resistance increased from 6.71 +/- 2.25 to 13.59 +/- 7.57 on days when subjects breathed filtered air alone and from 5.23 +/- 1.23 to 12.54 +/- 6.17 on days they breathed 0.25 ppm sulfur dioxide in filtered air. The increase in specific airway resistance on the 2 days was not significantly different. We conclude that 0.25 ppm sulfur dioxide may cause bronchoconstriction in freely breathing, heavily exercising subjects with asthma but that the effect is small and is largely overshadowed by bronchoconstrictor effect of exercise alone.

Objective: To more closely define the limit of sulfur dioxide concentration that causes bronchoconstriction in asthmatic persons, we measured the effects of 0.25 ppm sulfur dioxide on airway caliber in asthmatic subjects performing heavy exercise.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.25 ppm SO_2	5 min at rest 5 min during exercise	16 men 3 women	22-46 yrs	19	Asthmatic	N/A	N/A
0.25 ppm SO_2	5 min at rest 5 min heavy exercise			9 (chosen from above)			

Observations:

Inhalation of 0.25 ppm SO_2 by subjects during exercise at 750 kg m/min increased specific airway resistance only slightly more than did exercise alone. The change in specific airway resistance produced by exercise plus sulfur dioxide was significantly different from that produced by exercise alone ($p<0.05$).

SO_2 breathed during exercise at 1000 kg m/min did not increase specific airway resistance more than did exercise alone.
*Statistically significant

Author's Conclusions:

We conclude that 0.25 ppm sulfur dioxide may cause bronchoconstriction in freely breathing, heavily exercising subjects with asthma, but that the effect is small and is largely overshadowed by bronchoconstrictor effect of exercise alone.

Review – Assessment:

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-)Are the subjects a representative sample of the population to which extrapolations will be made? (+) Was the method of recruitment appropriate? (No info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+)Were subjects informed about the experimental risks? (No info) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+/-)Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+)Was a control group assayed? (+)Is the control regime appropriate?

	(+)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (+/-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+/-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+)Was individual data reported for each subject? (N/A)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+/-)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

19 subjects were exposed to 0.25 ppm SO_2 while undergoing heavy exercise. Inhalation of 0.25 ppm SO_2 by subjects during exercise at 750 kg m/min increased specific airway resistance only slightly more than did exercise alone. The change in specific airway resistance produced by exercise plus sulfur dioxide was significantly different from that produced by exercise alone ($p<0.05$). SO_2 breathed during exercise at 1000 kg m/min did not increase specific airway resistance

more than did exercise alone. The conclusion that 0.25ppm SO_2 is close to the threshold concentration capable of causing bronchoconstriction can only be applied to these young subjects with mild asthma. Also, only one temperature and relative humidity was studied. This study demonstrated that the concentration may cause bronchoconstriction, but this is reversible with rest and overshadowed by exercise-induced bronchoconstriction. This study provides support for human exposure guidelines for sensitive populations and the lifestyle stress that would have to be avoided, i.e. heavy physical labour when ambient concentrations are >0.25 ppm.

Confidence index:

Minor deficiencies pertaining to reagent source, follow-up of subjects slightly lower confidence in this study. The subject population is not representative of all asthmatics in the general population. Furthermore, subject response depends on the severity of the asthma and the second study which took place during exercise at 1000kg m/min was only conducted on 9 of the original subjects.

Review – Confidence index ranking:

Moderate

DRAFT

Author: Douglas RB. Coe JE.	**Internal ID: 121**
Title: The relative sensitivity of the human eye and lung to irritant gases.	
Year: 1987	
Abstract: Various concentrations of irritant gases were applied to the eyes of subjects through close fitting goggles and, in separate experiments, to the lungs via a mouthpiece. Eye response was detected subjectively, lung response was measured objectively through a plethysmograph and the threshold concentrations for no response were determined. For a number of gases tested (sulphur dioxide, ammonia, butan-2-one, pentan-2-one, pentan-3-one, 3 methyl butan-2-one, formaldehyde and acrolein), the threshold concentrations for the eyes was about 1 ½ times that for the lungs. Other irritants however, can be specific for certain organs.	
Objective: n/a	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
~3-60ppm	**a) eyes 15s** **b) lungs 10 breaths**	**n/a**	**m/a**	**n/a**	**n/a**	**n/a**	**a) eyes 5ppm** **b) lungs 1ppm**

Observations:

"For sulphur dioxide there is a physiological increase in tear production with exposure, returning to baseline within 15min. There is also a graded response (not seen with the subjective methodology above) which permits dose-response and threshold estimations. The threshold at the eye at 5ppm is still greater than that for the lung, 1ppm."
*Statistically significant

Author's Conclusions:

"There is no absolute reason why all organs should have the same sensitivity for irritants. The lung may be slightly the more sensitive organ, but for general, non-specific irritants there seems to be a considerable degree of co-sensitivty, which may be a useful adjunct to the practical problem of setting hygiene standards."

Review – Assessment:

A. Subjects:	±✎ Is the number of subjects sufficient to produce statistically significant results? -✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ±✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? ±✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?

B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? +/-✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? -✎ Was the duration of exposure appropriate to investigate the primary objective? -✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +/-✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined -✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✎ Were the subjects randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? ±✎ Is the control regime appropriate? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) -✎ Was the length of follow-up appropriate? -✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? ±✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	±✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported?

	±✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
G. Interpretations:	-✎ Was the original objective addressed? -✎ Are the results novel? -✎ Was the design of the study appropriate to investigate the primary objective? -✎ Were assumptions reasonable? -✎ Are the methods reproducible? ±✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The aim of this study was to investigate the irrative effects of a number of gases, vapors and aerosols to the lung and eye. The conclusions by the investigators state that for SO_2, the sensitivity of the eye is 1.5 times that for the lung. It is unclear what the clinical significance of this paper is from the text of the paper. However, the study does elucidate an interesting comparison regarding the sensitivity of various organs to various irritants, as such if the actual experimental results could be obtained would provide excellent support for human exposure guidelines.

Confidence index:

Although this paper was designed as a short communication, it was very poorly written. The objective of the study was unclear although the experimental seemed ambitious as it described the testing of 8 different gases. The experimental design, equipment and setup were vaguely described and no information about the subjects was given. The method for normalizing lung and eye response data was questionable at best. The SO_2 data appeared interesting for eye exposure but no supporting information about the data collection and analysis renders the observations invalid. The graded dose-response curve appears to be constructed with 2 points and no control, which is totally unacceptable.

Review – Confidence index ranking:

Low

Author: Folinsbee, L.G., Bedi, J.F., Horvath, S.M.	**Internal ID:** 122
Title: Pulmonary response to threshold levels of sulfur dioxide (1.0ppm) and ozone (0.3ppm)	
Year: 1985	
Abstract: We exposed 22 healthy adult nonsmoking male subjects for 2 h to filtered air, 1.0 ppm sulfur dioxide (SO_2), 0.3 ppm ozone (O_3), or the combination of 1.0 ppm SO_2 + 0.3 ppm O_3. We hypothesized that exposure to near-threshold concentrations of these pollutants would allow us to observe any interaction between the two pollutants that might have been masked by the more obvious response to the higher concentrations of O3 used in previous studies. Each subject alternated 30-min treadmill exercise with 10-min rest periods for the 2-h. The average exercise ventilation measured during the last 5 min of exercise was 38 l/min (BTPS). Forced expiratory maneuvers were performed before exposure and 5 min after each of the three exercise periods. Maximum voluntary ventilation, He dilution functional residual capacity, thoracic gas volume, and airway resistance were measured before and after the exposure. After O_3 exposure alone, forced expiratory measurements (FVC, $FEV_{1.0}$, and FEF_{25-75}%) were significantly decreased. The combined exposure to SO_2 + O_3 produced similar but smaller decreases in these measures. There were small but significant differences between the O_3 and the O_3 + SO_2 exposure for FVC, $FEV_{1.0}$, $FEV_{2.0}$, $FEV_{3.0}$, and $FEF_{25-75\%}$ at the end of the 2-h exposure. We conclude that, with these pollutant concentrations, there is no additive or synergistic effect of the two pollutants on pulmonary function	
Objective: We hypothesized that exposure of individuals to near-threshold levels of each pollutant would offer a better opportunity to demonstrate an additive, interactive, or synergistic effect if indeed one was present. Therefore, we studied the response of young male subjects to a combination of 0.3ppm O_3 and 1.0ppm SO_2 as well as to each gas individually.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
1. filtered air 2. 1 ppm SO_2 3. 0.3 ppm O_3 4. 1 ppm SO_2 + 0.3 ppm O_3	2 hours each (including 3 – 30 min exercise periods)	male	19-28 yrs	22	healthy	N/A	N/A

Observations:

In general, there were no important health-related differences between the effects of O_3 alone and the O_3 + SO_2. There was a small but significant difference between these exposures at minute 115 when examined by the a priori comparison. Pulmonary function parameters exhibited greater changes following O_3 exposure than following the combination O_3+SO_2 exposure. Pulmonary function parameters did not decrease across time in the filtered air or SO_2 exposures.
*Statistically significant

Author's Conclusions:

In summary, we found no evidence of a synergism between O_3 and SO_2. Indeed, our results would suggest that the effects of O_3 might be moderated slightly in the presence of SO_2. These findings, in conjunction with our two other studies of combination exposure to SO_2 and O_3 provide consistent evidence that the response to either pollutant is not aggravated in the presence of the other.

Review – Assessment:

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-) Are the subjects a representative sample of the population to which extrapolations will be made? (+) Was the method of recruitment appropriate? (No info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? (Yes) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+/-)Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+/-)Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (+/-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+)Is the control regime appropriate? (-)Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+)Was the length of follow-up appropriate? (+/-)Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (+/-)Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-

	response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+/-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (N/A)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (+/-)Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (N/A)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study attempted to determine the differences between exposure to either sulfur dioxide or ozone, or both gases together. The level of SO_2 exposure (1.0 ppm) was below the threshold response of humans as determined by airway resistance. Therefore, this paper provides support for a human exposure guideline below 1.0 ppm. In this study, there was no evidence of a synergism between O_3 and SO_2 at the concentrations tested (0.3 ppm and 1.0 ppm respectively). The potential interaction of O_3 and SO_2 is a concern because of the possibility on reactions that would result in a greater impact of these pollutants on the pulmonary systems.

Confidence index:

Information deficiencies as to reagent source, description and sensitivity of monitoring devices, and follow-up of subjects somewhat detracts from the confidence in this study. This study, by using 22 subjects was designed to investigate $SO_2 + O_3$ alone or in combination at different humidity levels. After the results showed that there was not significance in the different levels of humidity tested, the data was pooled. This was a valid conclusion and procedure. Although the subjects appeared to be relatively homogeneous as given by individual data and preliminary lung function testing, had a varied response for O_3 and SO_2. Effects were only seen after 115 min. or 5 min before the exposure period ended. It is apparent that the SO_2 concentration chosen to perform this study was too low (1.0 ppm) for this study group. Without a positive effect, it is difficult to assess the contribution of SO_2 to examine if the interactions are additive or synergistic. The study would have benefited by using asthmatics that would be more sensitive to both SO_2 and ozone. This study group would have also been more clinically relevant as pollution control guidelines are usually set to protect this group rather than normals. As such, the conclusion that there is no synergy is true only for this combination of concentrations of SO_2 and O_3 but it is unproven for higher SO_2 concentrations.

Review – Confidence index ranking:

Moderate

DRAFT

Author: Bethel RA. Sheppard D. Epstein J. Tam E. Nadel JA. Boushey HA.	**Internal ID: 123**

Title: Interaction of sulfur dioxide and dry cold air in causing bronchoconstriction in asthmatic subjects.

Year: 1984

Abstract: To determine whether sulfur dioxide and airway cooling and drying interact in causing bronchoconstriction in persons who have asthma, we measured specific airway resistance in seven asthmatic subjects before and after they performed voluntary eucapnic hyperpnea for 3 min breathing four different gas mixtures. The mixtures, which the subjects breathed through a mouthpiece in random order on 4 different days, were 1) humidified room-temperature air, 2) humidified room-temperature air containing 0.5 ppm SO$_2$, 3) cold dry air, and 4) cold dry air containing 0.5 ppm SO$_2$. Each subject breathed at a rate and depth known from preliminary studies to cause little or no bronchoconstriction when that subject inhaled 0.5 ppm SO$_2$ in humidified room-temperature air or cold dry air. When given independently in the blinded study, 0.5 ppm SO$_2$ or cold dry air again caused insignificant bronchoconstriction, but when given together the two stimuli caused significant bronchoconstriction, as indicated by an increase in specific airway resistance from 6.94 +/- 2.85 to 22.35 +/- 10.28 l X cmH2O X l-1 X s (mean +/- SD) (P less than 0.001). thus airway cooling and/or drying increases the bronchoconstriction induced by inhaled SO$_2$ in persons who have asthma. This increase suggests that persons who have asthma may be more sensitive to the bronchoconstrictor effects of ambient SO$_2$ in cold dry environments than in warm moist environments.

Objective: "…we sought to determine whether sulfur dioxide and cold dry interact in causing bronchoconstriction in persons who have asthma."

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.5ppm	**3min intervals sequential increasing breath rate**	**5M 2F**	**24-36**	**7**	**4 healthy** **3 airway obstruction**	**n/a**	**0.5ppm**

Observations:

"The control stimulus (humidified room temperature air), the SO2 stimulus (0.5ppm SO2) in humidified room-temperature air), and the cold dry air stimulus did not cause a significant increase in specific airway resistance; but 0.5ppm SO2 combined with cold dry air caused a considerable increase (from 6.94±2.85 to 22.35±10.28 l cmH$_2$0/l s. The combination of sulfur dioxide and cold dry air exacerbated the symptoms of shortness of breath and wheezing in six of the seven subjects."

*Statistically significant

Author's Conclusions:

"In conclusion, we have found that 0.5ppm SO$_2$ and cold dry air, two naturally occurring stimuli, interact positively in causing bronchoconstriction when inhaled through a mouthpiece by people who have asthma."

Review – Assessment:

A. Subjects:	+✏ Is the number of subjects sufficient to produce statistically significant results? +✏ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study?

	+✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? +✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? +✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✎ Were the subjects randomly assigned to exposure groups? +✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? +✎ Is the control regime appropriate? +✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? ±✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✎ Are the statistical methods used appropriate?

	+✓ Are confidence intervals reported? +✓ Did the investigator appropriately interpret the statistics? +✓ Are the observed trends statistically significant?
G. Interpretations:	+✓ Was the original objective addressed? +✓ Are the results novel? +✓ Was the design of the study appropriate to investigate the primary objective? +✓ Were assumptions reasonable? +✓ Are the methods reproducible? +✓ Did either the study design or the statistical analysis limit confounding variables? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the interaction between SO_2 and dry cold air in causing bronchoconstriction is asthmatics. The results show a marked increase in bronchoconstriction when asthmatics were exposed to SO_2 in dry cold conditions. The interaction was synergistic; bronchoconstriction was much greater than the effects of SO2+cold dry conditions alone. This study shows that clinically, asthmatics are more susceptible to SO2 exposures in cold, dry days. Also, the results have implications on the design of future studies as they clearly show the need for setting specific temperatures, humidity and the importance of measuring these factors during the experiment.

Confidence index:

The number of subjects used in this study was small (n=7) and little history was given on the severity of the asthma for each individual. Histamine challenge showed a wide range of general pulmonary function. In general the experiment design was logical and the investigators chose a unique exposure regime for the study. Each subject was pre-tested with both SO_2 and dry cold air to evaluate the ventilatory rate to reach the threshold of bronchoconstriction at a fixed concentration, temperature and humidity. A ventilatory rate was then chose to be just below threshold for both and pulmonary data collected for exposures of each alone and then in combination. The results were striking and effects were easily extracted from the statistical analysis. Surprisingly, the variation was not severe. Extrapolation of these specific results may not be possible, however the general conclusion that dry cold air enhances SO_2 bronchoconstriction (or vice-versa) is valid.

Review – Confidence index ranking:

Moderate to high

Author: Amdur MO	**Internal ID: 125**

Title: Effect of a combination of SO_2 and H_2SO_4 on guinea pigs

Year: 1954

Abstract: n/a

Objective: The objective of this paper is to examine the effects of independent and concurrent SO_2 and H_2SO_4 exposures on the lungs of guinea pigs.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
89ppm SO_2 and/or 8mg/m^3 H_2SO_4)	**8-16 hrs**	**Guinea pig**	**n/a**	**n/a**	**n/a**	**8: H2SO4+SO2** **4: SO2 alone** **12 total**	**n/a**	**n/a**	**n/a**

Observations:

After exposure to each of SO_2 alone and SO_2+H_2SO_4 weight of the guinea pigs dropped the day after exposure. Growth resumed quickly following the 8 hr SO_2 exposure, but was slow to resume after 8 hr exposure to SO_2 and H_2SO_4, and never did resume following a 16hr exposure to both substances.
Few signs of respiratory stress were witnessed following exposure to SO_2 alone, but extensive distress was evident following the combined exposure.
Lung pathology following SO_2 exposure included pinpoint hemorrhages and occasional small areas of consolidation, but the damage was not severe. In contrast, following the combined SO_2 + H_2SO_4 exposure, large areas of complete consolidation and hepatization were evident. Ill health of the animals was likely related to this extensive lung damage.

*Statistically significant

Author's Conclusions:

"It has been shown that for 8mg per cu. m. of sulfuric acid mist and 89ppm of SO_2 given in combination to guinea pigs the effect on growth, lung pathology, and respiration are much more marked than would be predicted from use of either alone."

Review – Assessment:

A. Test animals:	±✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* -✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age

	☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex -d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✏ Were the chosen exposure levels appropriate to investigate the primary objective? -✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ±✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? -✏ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? +✏ Was evidence provided to justify the dosing regime? -✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	-✏ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✏ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✏ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	-✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)*

	-✏ Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✏ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? -✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) -✏ Was a control group assayed? -✏ Is the control regime appropriate? -✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? -✏ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +/-✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? -✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? -✏ Was the timing and frequency of assessment logical? +✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✏ Was individual data reported for each test animal?
G. Data analysis:	-✏ Are the statistical methods used appropriate? -✏ Are confidence intervals reported? ±✏ Did the investigator appropriately interpret the statistics? ±✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? -✏ Was the design of the study appropriate to investigate the primary objective? -✏ Were assumptions reasonable? +✏ Are the methods reproducible? -✏ Did either the study design or the statistical analysis limit confounding variables? +✏ Do the conclusions make biological sense? +-✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings demonstrate that even high levels of SO_2 (89ppm) do not appear to have major effects in guinea pigs exposed for long periods of time, aside from an initial decrease in weight, and some minor pathology in the lungs. Similar results are witnessed for H_2SO_4 alone. In contrast, combined SO_2 and H_2SO_4 exposures show major pathology, and appear to demonstrate synergism of effect. This might have implications for humans exposed to both SO_2 and H_2SO_4; even though individual levels of these agents may be within limits, negative effects may still be witnessed. This study was probably one of the first papers to show the nature of damage of H_2SO_4 and SO_2 alone and in combination.

Confidence index:

This was a very old paper, which described a crude procedure for evaluating the effects of H_2SO_4 and SO_2. The conclusions that the combination of the two gases is synergistic is invalid as the author did not quantify the damage due to each gas. The increased lung damage may have been an additive effect as the combination of the two gases was done at concentrations matching the experiments performed alone. The anecdotal data given by the morphology study is probably useful for clinical studies, however the subjective nature of the damage makes it difficult to report in such a way to give conclusive evidence.

Review – Confidence index ranking:

Low to moderate

Author: Suzuki, T	**Internal ID:** 126
Title: Effect of exposure to O_3, SO_2 and NO_2 upon the lung histamine content of guinea pigs	
Year: 1969	
Abstract: The lung histamine contents of air control and O_3, SO_2, No_2 exposed guinea pigs were compared, and the water content of the lungs was estimated in each group. It was noted that the mean histamine content of the lungs of animals exposed to low and high concentrations of ozone was below the mean of the control group. Lung edema was produced by both low and high concentrations of ozone exposure. Massive lung edema was also produced in the lungs of guinea pigs exposed to a high concentration of NO_2, while there was no significant change in the mean histamine content of the lungs. Using an apparatus for perfusion of isolated lungs of guinea pigs, a large amount of histamine was found to be contained in the perfusion out-flow collected during 0 to 90 minutes from O_3 ventilated lungs.	
Objective: The lung histamine contents of air control and O_3, SO_2, NO_2 exposed guinea pigs were compared, and the water content of the lungs was estimated in each group.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
10, 50 ppm	3 hours/ once	Guinea pigs	Random-bred	Na	Male	6 at each concentration total = 12	Unknown	No observed adverse effect level	Lowest observed adverse effect level

Observations:

It was noted that the mean histamine content of the lungs of animals exposed to low and high concentrations of ozone was below the mean of the control group. Lung edema was produced by both low and high concentrations of ozone exposure. Massive lung edema was also produced in the lungs of guinea pigs exposed to a high concentration of NO_2, while there was no significant change in the mean histamine content of the lungs. Using an apparatus for perfusion of isolated lungs of guinea pigs, a large amount of histamine was found to be contained in the perfusion out-flow collected during 0 to 90 minutes from O_3 ventilated lungs. "SO_2 exposure, both in low and high concentrations of the gas, had no effect on the water and histamine content of the lungs of the animals."

*Statistically significant

Author's Conclusions:

The guinea pigs exposed to SO_2 did not show any change in the lung water content and histamine content possible because SO_2 differs markedly from O_3 and NO_3 in that its acutely irritant effects ware confided to the upper respiratory tract.

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex (+) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation *Guideline: Below 20% variation* (-) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+/-) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+/-) Was the frequency of administration appropriate to investigate the primary objective?

	(-) Was evidence provided to justify the dosing regime? (N/A) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (N/A) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (+/-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+/-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (N/A) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+/-) Were appropriate endpoints examined?

	(+/-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+/-) Were accepted assessment methods employed? (+/-) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+/-) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+/-) Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?
H. Interpretations:	(+/-) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (+/-) Were assumptions reasonable? (+/-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+/-) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the effect of SO_2 and other gases on the histamine content of guinea pigs. Histamine has long been known to be a recognized participant in the inflammatory process. The results show that SO_2 have little effect on the histamine content or water content in the lung. The authors conclude that considering the effects of O_3 and NO_2, that SO_2 effects are confined to the upper respiratory tract. As a more general statement, it is obvious that SO_2 has a different mechanism of action of toxicity compared to the other gases. This study, other than lacking one more exposure gradient, could have been quite useful to human exposure guidelines, especially in the comparison to other irritant gases. However, the deficiencies noted below severely hinder extrapolation of this data to human models.

Confidence index:

This study employed only two dosage levels of SO_2. It is not clear if the same 6 guinea pigs were used for each exposure and there is no justification for the exposure levels chosen. Lack of information as to source of animals, pre test health, method of assigning to test groups, diet, source of reagents, monitoring equipment sensitivity all reduce confidence in this study. Furthermore, the weight variation among the guinea pigs was too large.

Review – Confidence index ranking:

Low

DRAFT

Author: Wakabayashi M. Bang BG. Bang FB.	**Internal ID: 129**
Title: Mucociliary transport in chickens infected with Newcastle disease virus and exposed to sulfur dioxide.	
Year: 1977	
Abstract: Mucociliary transport was studied in the nasal mucous membranes and sinuses of 3-week-old chickens which were either exposed to sulfur dioxide (SO_2), infected intranasally with the mesogenic strain of Newcastle disease virus (NDV), or exposed to SO_2 after NDV infection. A newly developed apparatus was used to follow intranasal transport rates over time in the same animal, and to follow sinus transport rates over time in a separate group of animals. Intermittent exposure to concentrations of 1.4-66.0 ppm SO2 produced peaks of increased intranasal transport time, with intervening recovery periods. This suggests a homeostatic mechanism. Transport was also decelerated in the sinus when concentrations of SO_2 were above 10 ppm. NDV infection produced decelerated intranasal transport rates but did not decelerate sinus rates. Combined NDV and SO_2 interacted to produced persistent deceleration of the intransal transport rate. In the sinus, the combination seemed to conteract the decelerating effect of SO_2 alone, suggesting a separate mechanism of homeostasis.	
Objective: "The primary purpose of this study was to investigate the effect of SO_2 alone, viral infection alone, and their synergistic effects on mucociliary transport activity in the nasal turbinates and sinuses of live animals, under completely physiological conditions."	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
1.4-66.0ppm	**Intermittent for 16hrs/day for 7 days**	**chicken**	**leghorn**	**14-30days**	**n/a**	**45**	**Healthy and Newcastle virus**	**n/a**	**1.4ppm**

Observations:

"When the animals were exposed to SO_2 at 1.4±1.0ppm, the turbinate (clearance rate) times were elevated slightly even after one exposure; the times returned to normal values by the third day, then again increased gradually. Just after the last (seventh) exposure, mucociliary transport action was almost stopped. However, 48hr after SO_2 exposure ceased and the chickens were left in the room environment, clearance times approached normal values. At a concentration of 12.0±5.2ppm SO_2, turbinate time after one exposure showed a remarkable increase."

"…after initial exposure to very high concentrations of SO_2 (66.0±12.0ppm), mucociliary activity almost completely stopped, recovered during several subsequent intervals of exposure, decelerated again, and recovered once again during successive exposures… Thus, patterns of turbinate times after SO_2 exposure differed according to concentrations of SO_2 and also varied in individual chickens. However, each pattern was basically biphasic, since recovery phenomena were observed at all three concentrations during the time of exposure to SO_2."

Sinus time (mucociliary transport rate) was not affected at 1.4ppm, increased after the 3rd exposure to 12.0ppm SO_2, and was seriously slowed at 66.0ppm. Combined exposure to the virus and SO_2 increased clearance rate time, while the combined exposure had an antagonistic effect on mucociliary activity.

*Statistically significant

Author's Conclusions:

"What we have learned from the interaction of SO_2 and intranasal virus infection in the present model cannot, of course be interpreted as mirroring the natural occurrence of SO_2 and myxovirus infection in the human environment. The results strongly suggest, however, that elevated SO_2 levels may seriously impair normal defenses against nasally inhaled respiratory agents, and that interaction between the two factors might have especially serious consequences in selected individuals in a population."

Review – Assessment:

A. Test animals:	±✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? -a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +/-c) Sex +d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* +f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers

	appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* +✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* +✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* +✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? -✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +/-✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?

G. Data analysis:	+/-✓ Are the statistical methods used appropriate? -✓ Are confidence intervals reported? +/-✓ Did the investigator appropriately interpret the statistics? +/✓ Are the observed trends statistically significant?
H. Interpretations:	+✓ Was the original objective addressed? +✓ Are the results novel? +✓ Was the design of the study appropriate to investigate the primary objective? +✓ Were assumptions reasonable? +✓ Are the methods reproducible? -✓ Did either the study design or the statistical analysis limit confounding variables? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the effects of SO_2 and viral infection of the nasal tract on muscociliary transport in chickens. SO_2, even at low concentrations affected the function of the mucosa in chickens. This effect appeared to be immediate, does-related and continued as the SO_2 exposure was continued. Nasal infection with a virus (NDV) along with SO_2 tended to cancel the increase in sinus times produced by high concentration SO_2. The results suggest that elevated levels of SO_2 may seriously impair normal defense against nasally inhaled respiratory agents. This study has little applicability to human exposure guidelines as it is examining mechanisms that may or may not be applicable to humans from birds which have a completely different respiratory regime. No justification of the use of this animal subject was provided.

Confidence index:

There were many limitations of this study. The animals in this study were not fully described in the text and critical variables such as body mass and gender were not disclosed. The experimental procedure was not logical and designed in such a way that exposure conditions could not be compared. Treatments with SO_2 were at 1.4 12 and 66 ppm, however, SO_2 + virus were done at SO_2 concentrations of 8.2 and 10.8. Also, SO_2 exposures were performed when the chickens were 21 days old compared to 15 days old for the combined study. Lastly, there was no clear justification why chickens were used for this study. Chickens do have relatively simple nasal structure compared to humans, which make this type of study easier, however, without accounting for complex absorption and removal mechanisms of the human nose, extrapolation of the data is not possible.

Review – Confidence index ranking:

Low to Moderate

Author: Riechelmann, H., Maurer, J., Kienast, K., Hafner, B., Mann, W.J.	**Internal ID:** 132
Title: Respiratory Epithelium Exposed to Sulfur Dioxide-Functional and Ultrastructural Alterations	
Year: 1995	
Abstract: The value of morphological investigations of airway mucosa should be compared to a functional method when estimating the toxicity of airborne pollutants. In 34 guinea pig tracheas, mucociliary activity was measured using a modified light beam reflex method before and following exposure to sulfur dioxide for 30 minutes in concentrations ranging between 7.5 and 37.5 mg/m3. Exposure to air served as a control. Simultaneously, specimens were taken for light and electron microscopy. Mucociliary activity decreased from 8.4 +/- 2.9 Hz (control exposure) to 4.0 +/- 2.9 Hz following exposure to 7.5 mg/m3, to 3.4 +/- 2.7 Hz at 15 mg/m3 sulfur dioxide, to 1.8 +/- 2.2 Hz at 22.5 mg/m3 sulfur dioxide, to 1.5 +/- 1.8 Hz at 30 mg/m3 sulfur dioxide, and to 2.0 +/- 1.2 Hz at 37.5 mg/m3 sulfur dioxide, respectively (P < .01). Despite a 56% decrease in mucociliary activity, only minor morphological alterations were observed following exposure to 7.5 mg/m3 sulfur dioxide. However, following exposure to 15 mg/m3 sulfur dioxide or higher, structural alterations of respiratory mucosa such as epithelial sloughing, intracellular edema and mitochondrial swelling, widened intercellular space, and ciliary cytoplasmic extrusions were found. Thus measurement of mucociliary activity proved to be a more sensitive indicator of airway toxicity than structural investigations alone.	
Objective: The aim of this study was to correlate functional alterations of the mucociliary system with morphological changes of mucosal specimens following exposure to various sulfur dioxide concentrations.	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
7.5-37.5 mg/m^3	30 minutes	Guinea pigs	No info	No info	No info	34	No info	N/A	N/A

Observations:

Exposure to 7.5 mg/m^3 Sulfur Dioxide

Mucociliary activity of trachea specimens was reduced to 4.0±1.1 Hz ($p<.01$).

Exposure to 15 and 22.5 mg/m^3 Sulfur Dioxide

Mucociliary activity was decreased to 3.4±2.7 Hz following exposure to 15 mg/m^3 sulfur dioxide and to 1.8±2.2 Hz following exposure to 22.5 mg/m^3 sulfur dioxide.

Exposure to 30 and 37.5 mg/m^3 Sulfur Dioxide

Mucociliary activity was further reduced following exposure to 30mg/m^3 sulfur dioxide (1.5±1.8 Hz) and 37.5 mg/m^3 sulfur dioxide (2.0±1.2 Hz).

*Statistically significant

Author's Conclusions:

Although the concentrations used in these short term toxicity experiments are approximately 25 times higher than ambient sulfur dioxide concentrations in

polluted areas, it is likely that these alterations are important pathophysiological factors and may lead to an increased incidence of upper air way diseases in polluted areas. Thus measurement of mucociliary activity proved to be a more sensitive indicator of air way toxicity than structural investigations alone.

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (-)c) Sex (-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (-) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.*

	(+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+/-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-)Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (+/-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (+) Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? (+) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*

F. Data collection:	(+)Were appropriate endpoints examined? (-)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+/-) Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (+)Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the use of morphological investigations and mucociliary function to evaluate exposure to SO_2. The aim was not to derive toxicological or physiological effects of SO_2 but to evaluate a technique for monitoring effects from toxic gases. The measurement of mucociliary activity appeared to be the better indicator of the toxic effects of sulfur dioxide. For example, a 30-minute exposure to 7.5 mg/m^3 sulfur dioxide resulted in a 56% decrease in mucociliary activity when compared with the control exposure. After exposure to 15 mg/m^3 or higher concentrations of SO_2, morphological alterations occurred together with a pronounced reduction of mucociliary activity. However, there are many problems with the study design that prevent any meaningful conclusions being made.

Confidence index:

Although the aim of the study was to investigate a new method, information about the pool of animals used was still necessary. Information such as breed, weight variation, health and sex were not given. These factors could potentially lead to variables that could confound the comparison. Indeed, the exposure of the trachea at higher SO_2 levels gave large variability in the measurement of mucociliary activity. The utility of the technique is questionable as exposure of SO_2 was performed after the dissection of the trachea. It was not stated if this was necessary to perform the gathering of data. If so the method may not be able to provide useful data as it would be difficult to separate effects from SO2 exposure and death of the animal. Also, experiments involving temporal effects or prolong exposures would not be possible.

Review – Confidence index ranking:

Low

DRAFT

Author: Riedel F. Kramer M. Scheibenbogen C. Rieger CH.	**Internal ID: 133**

Title: Effects of SO_2 exposure on allergic sensitization in the guinea pig.

Year: 1988

Abstract: The effect of sulfur dioxide (SO_2) exposure on local bronchial sensitization to inhaled antigen was studied in the guinea pig. Exposure to SO_2 (0.1 to 16.6 ppm) was performed in a 20 L exposure chamber for 8 hours on 5 consecutive days, while temperature, moisture, and concentration of SO_2 were monitored and kept constant. SO_2 concentrations were measured hourly by Schiff's reaction. On the last 3 days, SO_2 exposure was followed by inhalation of nebulized ovalbumin (OA) for 45 minutes. One week later, specific bronchial provocation with inhaled OA (0.1%) followed by plethysmographic measurements of airway obstruction were performed every 2 days during a 2-week period. Specific antibodies against OA were measured in serum and bronchoalveolar fluid by a direct enzyme immunoassay. The SO_2-exposed group (N = 17) demonstrated 67% to 100% positive bronchial reactions to inhaled OA, depending on the concentration of SO_2, whereas the control group without previous SO2 exposure (N = 14) demonstrated bronchial reactions in only one animal (7%: p less than 0.05). The degree of bronchial obstruction was significantly higher in the exposed group, compared to the control group, for all SO_2 concentrations (p less than 0.05). OA-specific antibodies in serum and bronchoalveolar fluid increased in SO_2-exposed groups significantly, compared to the control group (p less than 0.05). It is concluded from these results that exposure to SO_2 in low and medium concentrations can facilitate local allergic sensitization in the guinea pig.

Objective: "To elucidate further the association between low levels of air pollution and inhalative allergic sensitization, we investigated the effect of low and medium levels of SO_2 on the local allergic sensitization to inhaled OA with lung physiologic and immunologic parameters in guinea pigs."

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
0.1-16.6ppm	**8hrs/day for 5 consecutive days**	**Guinea pig**	**Perlbright-White**	**300-350 grams**	**female**	**31**	**Healthy**	**n/a**	**0.1ppm**

Observations:

"Although individual animal responses varied considerably, all animals reacted at higher SO_2 concentrations (4.3ppm) with more severe airway obstruction (TA), indicateing more pronounced sensitization to OA (ovalbumin). A similar pattern was demonstrated for the immunologic parameters of sensitization with increased specific antibodies in serum and bronchoalveolar fluid. No further enhancement was noticed after SO_2 concentrations of 16.6ppm, neither in pulmonary function, nor in immunologic parameters."

*Statistically significant

Author's Conclusions:

"…we conclude that short exposure, even to low concentrations of SO_2 enhance allergic inhalative sensitization in our animal model with some effect already at concentrations of 0.1ppm of SO_2. This level of exposure lies below the allowed average day concentration of National Ambient Air Quality Standards (clean air act for 1970) for SO_2 ($365 \mu g/m^3$; ~0.14ppm) in the United States and below the proposed federal hourly standard of 0.5ppm of SO_2. Further studies with other irritant gases and longer exposure times are under present investigation."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex +d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? -✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.*

	±✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* ±✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✏ Was a full description of the monitoring devices provided? +✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✏ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? -✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? -✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? +✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✏ Was individual data reported for each test animal?
G. Data analysis:	+✏ Are the statistical methods used appropriate? -✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective?

	+✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the allergic sensitization of guinea pigs with SO_2. The results show that low concentrations of SO_2 (0.1 ppm) are able to sensitize guinea pigs to ovalbumin (OA) as indicated by increased IgG, IgG1 and anti-OA. The responses appear to be dose-responsive; increased SO_2 exposure caused an increase in sensitization while leveled off at higher concentrations. The clinical importance of this study is that a chronic low dose of SO_2 is able to produce an immunological response.

Confidence index

The number of animals used in this study was significant and adequate information was given about the animals. The exposure schedule was not as well detailed. For immunological studies such as this one, factors such as acclimatization periods, diet, lighting, temperature etc become important but were not disclosed. Immunosuppression is a non-specific effect and animal stress is a strong inducer of this variable. It appeared that the data was analyzed correctly, however, tabulation of the results so that exposure conditions and effects would have made the results clearer. Although the variance of each test group could be evaluated graphically, a numeric representation of the standard deviation (in a table) would have aided the interpretation. Regardless, the data was quite striking, and SO_2 clearly had an effect on sensitization.

Review – Confidence index ranking:

Moderate to high

Author: Trimpe, K.L., Weiss, H., Zwilling, B.S. **Internal ID:** 134

Title: The effect of SO_2 on the Clearance of *Listeria monocytogenes* from the Lungs of Emphysematous Hamsters

Year: 1986

Abstract: The effect of sulphur dioxide on the clearance of Listeria monocytogenes from normal and emphysematous hamsters was assessed by measuring the number of colony forming units recovered from whole lung homogenates. Continuous exposure to SO_2 after intratracheal instillation of Listeria significantly altered the clearance of viable bacteria from the lungs of emphysematous but not normal hamsters. Preexposure of hamsters to SO_2 for 2 weeks prior to respiratory infection had similar effects. The emphysematous hamsters exposed to SO_2 had a lower average number of Listeria in the lungs after the first week of infection than control groups. This effect appears to result from the combined influence of the SO_2, the Listeria infection, and the emphysematous condition within the lungs.

Objective: To assess the effect of SO_2 exposure on the clearance of bacteria from the lungs of animals with a form of chronic obstructive lung disease.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
27±3 ppm SO_2	Not clearly stated. Approximately 35 days of continuous exposure	Male Syrian golden hamsters	LSH/LAK	7-10 months	Male	**Not given**	**No informaiton**	N/A	N/A

Observations:

Effect of SO_2

The effect of sulfur dioxide exposure on the clearance rate of *L. monocytogenes* from emphysematous hamster lungs was assessed. There was no differecn in the rate of clearance of the bacteria from the lungs ofnormal or emphysemattous hamsters breathing filtered room air or SO_2 during the first 7 days.

The number of *L.monocytogenes* recoverable from the lungs of emphysematous hamsters exposed to SO_2 was significantly ($P<0.05$) less than that from the untreated or emphysematours animals.

The number of Listeria recovered from normal hamsters exposued to SO_2 also exhibited a moderate decline approaching the low level of Listeria recovered from the SO_2-exposed, emphysematous hamsters; however, this decrease was not statistically different from hamsters breathing filtered air.

*Statistically significant

Author's Conclusions:

Continuous exposure to SO_2 after intratracheal instillation of Listeria significantly altered the clearance of viable bacteria from the lungs of emphysematours but not of normal hamsters. Preexposure of hamsters to SO_2 for 2 weeks prior to respiratory infection with *L. monocytogenes* did not alter the clearance rates compared to animals expsoed to SO_2 beginning at the time of infection. After the first week of infection, the emphysematour hamsters exposed to SO_2 contained an averzge number of Listeria in the lungs which was significantly lower than that in the control groups. This trend may reut from the combined influence of the SO_2 exposure, the Listeria infection, and the emphysematous condition within the lungs.

Review – Assessment:

A. Test animals:	(-)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation *Guideline: Below 20% variation* (+) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined?

	(-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (+/-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water May be withheld optionally.*
D. Equipment:	(-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (-) Were test animals randomly assigned to exposure groups? (N/A)Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random

	Assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(-)Were appropriate endpoints examined? (-)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+/-)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (-)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the effect of SO_2 on the clearance of a bacterial infection (L monocytogenes). The results show that exposure to SO_2 at 27 ppm decreased clearance of Listeria after 7 days of exposure. The relevance of this study is in question as it is unknown if the decrease in bacteria was due to the physiological effects of SO_2 or the toxicity of SO_2 to the bacteria. This study does not provide any support or evidence for human exposure guidelines.

Confidence index:

This study was very poorly reported. The experimental setup and protocol were missing key procedural aspects such as temperature, humidity, light conditions, food/water, animals/exposure group etc. All of these factors would have a pronounced effect on the results. Regardless, the data is questionable at best due to the lack of statistical analysis. Data was present as graphs and there was no variability data and no mention of the number of observations/ point. No tests of significance was performed.

Review – Confidence index ranking:

Low

Author: Ukai K. Bang BG. Bang FB. **Internal ID: 137**

Title: Effect of infection and SO_2 exposure on nasal and paranasal mucociliary clearance in intact chickens.

Year: 1984

Abstract: The hypothesis that homeostatic control mechanisms control mucociliary function in ciliated mucous membrane was tested. Nasal mucociliary transport rates were recorded in chickens in vivo at successive intervals during exposure to SO_2 or after inoculation with Newcastle disease virus (NDV), or both. Either agent alone caused deceleration of the turbinate clearance. However, when SO_2 exposure was limited to one nasal fossa and turbinate mucociliary rates were determined in the unexposed and infected side, the two acted antagonistically and yielded approximately normal rates. Exposure of the nasal mucosae to SO_2 caused decreased rates of sinus clearance, while NDV infection of nasal membranes induced increased rates of sinus clearance. Exposure of nasal mucosae to both agents acted antagonistically to effect rates of sinus clearance in normal ranges. These data support the idea of homeostasis.

Objective: "The present paper reports further investigation of homeostatic mechanisms in nasal and paranasal mucociliary clearance in chickens exposed to either SO_2 or NDV, or to both these agents concomitantly."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
18-40ppm	**1 hr/day for 7 days**	**Chicken**	**White Leghorn**	**Newly hatched**	**n/a**	**~7 difficult to determine**	**Healthy & Newcastle virus**	**n/a**	**n/a**

Observations:

"The first experiment in which the interaction of SO_2 and infection was studied was designed to determine whether the previously reported slowing of turbinate clearance time produced by NDV would be changed if the SO_2 exposure was limited to the side opposite the infection…The usual decrease in transport rate was obtained in the infected side which had not had SO_2 exposure, but when the contralateral side was exposed to SO_2, the slowing was less apparent."
"Finally, when a comparison was made of the sinus clearance time for birds receiving brief intense SO_2 exposure, with and without NDV infection, it was clear that sinus clearance rates were not greatly decreased in birds infected with NDV."

*Statistically significant

Author's Conclusions:

"Results of these experiments support the concept of homeostasis. There were two main findings:

1) Effects of unilateral inhalation of SO_2 on turbinate clearance from the contralateral NDV-infected nasal membranes provided specific evidence of homeostasis response.
2) SO_2 alone induced deceleration of sinus clearance, but concomitant infection with NDV neutralized this effect."

Review – Assessment:

A. Test animals:	±✏ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* -✏ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex +d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✏ Were the chosen exposure levels appropriate to investigate the primary objective? -✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ±✏ Were both nominal and actual exposure concentrations recorded? -✏ Was the duration of exposure appropriate to investigate the primary objective?+-✏ Was the duration of exposure precisely defined? -✏ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +/-✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? +✏ Was evidence provided to justify the dosing regime? -✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	-✏ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✏ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✏ Was the manner by which animals were grouped appropriate?

	☐ *Guideline: Animals may be grouped by sex, or individually.* -✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* -✏ Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✏ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? -✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) -✏ Was a control group assayed? ±✏ Is the control regime appropriate? -✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? -✏ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? -✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✏ Was individual data reported for each test animal?
G. Data analysis:	+✏ Are the statistical methods used appropriate? ±✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel?

	±✏ Was the design of the study appropriate to investigate the primary objective? -✏ Were assumptions reasonable? -✏ Are the methods reproducible? +✏ Did either the study design or the statistical analysis limit confounding variables? -✏ Do the conclusions make biological sense? -✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the effect of viral infection and SO_2 exposure on nasal mucociliary clearance in chickens. The results showed that SO_2 alone induced deceleration of sinus clearance but this effect was reversed in the presence of a viral infection. This data supports the concept of homeostatsis.

It is unclear what the objective of this research was. The conclusion that homeostasis occurs in the regulation of mucociliary transport to stresses such as SO_2 or NDV infection can only be made for this specific case with chickens. It would be difficult to extrapolate the results from this model to others, especially the human model.

Confidence index:

It was difficult to assess the experimental design of this study as most of the experimental section was referenced to another paper. Limited information about the animals was given and it appeared that no screening was performed. For this reason, confounding variables are likely. Exposure conditions were not justified for the chronic exposure study. The procedure of taping a nostril to control SO_2 exposure is questionable; it is expected that SO_2 may be able to permeate locations of the blocked nostril through oral breathing. The graphs were confusing and although effects seemed to be significant, no statistical analysis was performed to prove such significance.

Review – Confidence index ranking:

low

Author: Ukai, K., Bang, B.G., Bang, F.B.	**Internal ID:** 138
Title: Effect of SO_2 exposure on nasal mucociliary clearance in intact chickens	
Year:1983	
Abstract: The time required for mucociliary clearance from the chicken nasal turbinate and from the maxillary sinus was investigated in individual animals by using a newly designed plastic holder for the experimental animals. Determined in this way were: 1) the effect of SO_2 exposure on sinus and turbinate clearance time, 2) the effect of the nerve blocking drugs atropine, scopolamine, reserpine, and propranolol on turbinate clearance time, and 3) the effect of these nerve blockers on clearance rates in chickens exposed to SO_2. Turbinate mucociliary clearance was measured at 5 intervals per day, during 1 to 7 hr after exposure, for 7 consecutive days. Sinus clearance time was measured twice-daily 1 to 4 hr after exposure. Turbinate clearance time in birds exposed to 6 ppm, and sinus clearance time in birds exposed to 40 ppm intermittently for 2 consecutive days both increased strikingly as a direct effect of SO_2 exposure. However, continuous exposure to 6 ppm of SO_2 during 16 hr per day for 7 consecutive days produced double peaks of increased turbinate clearance time with intervening recovery periods, suggesting an intranasal mucociliary homeostatic response. In individual animals, 26 of 35 animals (75%) exposed to 5 ppm, and 5 of 10 animals (50%) exposed to 20 ppm continuously during 16 hr per day for 7 consecutive days showed the same patterns. Reserpine and propranolol, which are sympatholytic agents, produced decelerated intranasal transport rates. Atropine and scopolamine, which are parasympatholytic agents, did not affect clearance rates. These nerve blockers, however, blocked the biphasic recovery pattern due to SO_2 exposure. This blocking effect was statistically significant for atropine and reserpine 1 hr after injection	
Objective: The purpose of this study is to investigate the effect of SO_2 exposure on intranasal mucociliary clearance by using a newly designed plastic chicken holder which makes it possible to monitor many sequential tests of mucociliary clearance rates in individual animals by direct vision.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
Varied from 4ppm-40ppm	Three experimental groups: 1. those receiving intermittent exposure of 1hr for 4x/d on 2 consecutive days 2. those receiving continuous exposure during 16hr/d for 7 consecutive days 3. those treated with nerve blocker once daily for 7 consecutive days as soon as	White Leghorn chickens	No info	3 wks of age for most of experiments	Presumably female, but not stated	327	No info	N/A	N/A

	exposure of SO_2 was withdrawn								

Observations:

Turbinate clearance time in birds exposed to 6 ppm, and sinus clearance time in birds exposed to 40 ppm intermittently for 2 consecutive days both increased strikingly as a direct effect of SO_2 exposure. However, continuous exposure to 6 ppm of SO2 during 16 hr per day for 7 consecutive days produced double peaks of increased turbinate clearance time with intervening recovery periods, suggesting an intranasal mucociliary homeostatic response. In individual animals, 26 of 35 animals (75%) exposed to 5 ppm, and 5 of 10 animals (50%) exposed to 20 ppm continuously during 16 hr per day for 7 consecutive days showed the same patterns. Reserpine and propranolol, which are sympatholytic agents, produced decelerated intranasal transport rates. Atropine and scopolamine, which are parasympatholytic agents, did not affect clearance rates. These nerve blockers, however, blocked the biphasic recovery pattern due to SO_2 exposure. This blocking effect was statistically significant for atropine and reserpine 1 hr after injection.
*Statistically significant

Author's Conclusions:

The chicken nasal and paranasal sinus mucous membranes have proved to be excellent models in which to study mucociliary response, including homeostasis. We suggest that the apparent homeostasis following SO_2 exposure may be regulated by reflex activity of parasympathetic and probably also of sympathetic nerves.

Review – Assessment:

A. Test animals:	(-)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (-)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (-)c) Sex (-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (-) f) Source

	Guideline: An established breeding facility or educational institution
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (-)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*

D. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+/-)Were accepted assessment methods employed? (+/-)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+/-) Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary

	objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+/-) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study investigated the nasal mucociliary clearance in chickens exposed to SO_2. The result show that clearance increased dramatically in birds exposed to 40 ppm SO_2 intermittently for 2 days. Continuous exposure at 6 ppm showed increases in clearance followed by periods of recovery. When birds were treated with sympatholyic agents (reserpine and propranolol) clearance was decelerated. Atropine and scopolamine (parasympatholytic agents) did not effect clearance. However, these nerve agents were able to block the recovery aspect of chronic SO_2 exposure. This data shows the mucociliary homeostasis response to SO_2. There were no statistically significant or biologically significant findings in this study. Many of the results were referred to as striking, but there is no mention of significance.

Confidence index:

The total number of birds used in this study was high, however the screening of the test birds appeared to be non-existent. This can be observed from the large variation in the collected data. It is curious that while a large number of birds were used, a number of studies report small sample sizes. The effect of short high dose SO_2 for days used only 3 chickens. Other experiments show n=6 and n=10 which are marginal to produce a significant response. The experimental procedure was not detailed in the text. Many aspects such as temperature, humidity, light cycle, diet were not given. These factors may have had a pronounced effect on homeostasis if the birds were stressed in some manner. The conclusion given by the authors is based on the bi-phasic pattern from the chronic exposure of SO_2. Furthermore, the authors state that the SO_2 exposure system has been reported previously, but there is no reference.

Review – Confidence index ranking:

Low

Author: Murray, F.J., Schwetz, B.A., Crawford, A.A., Henck, J.W., Quast, J.F., Staples, R.E.

Internal ID: 140

Title: Embryotoxicity of Inhaled Sulfur Dioxide and Carbon Monoxide in Mice and Rabbits

Year: 1979

Abstract: The embryotoxic and teratogenic potential of sulfur dioxide (SO_2) was evaluated in CF-1 and New Zealand rabbits exposed to SO_2 alone or in combination with carbon monoxide (CO). The animals inhaled filtered room air (controls), SO_2 (mice, 25 ppm; rabbits, 70 ppm), or SO_2 plus CO (250 ppm) for 7 hr/day from days 6 through 15 (mice) and from days 6 through 18 (rabbits) of gestation. In both species, inhalation of SO_2 resulted in slight toxicity in the dams and an increased incidence of minor skeletal variants among their offspring; exposure to the combination did not potentiate the increased incidence of these variants. A teratogenic effect was not discerned in either mice or rabbits exposed to SO_2 alone or in combination with carbon monoxide, but the fetuses of mice exposed to the combination were significantly smaller than those exposed only to SO_2 exposure. This blocking effect was statistically significant for atropine and reserpine 1 hr after injection

Objective: The purpose of the studies described in this report was to evaluate the potential teratogenicity of inhaled SO_2 in mice and rabbits. To evaluate the potential effects of inhaled SO_2 to evaluate the potential effects of inhaled SO_2 when given in combination with carbon monoxide (CO), another common atmospheric pollutant.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
Filtered air 25 ppm SO_2 25 ppm SO_2 + 250 ppm CO	7hr/d, Days 6 thru 15 of gestation	Mice	CF-1	unknown	Female	40 32 35	unknown	unknown	unknown
filtered air 70 ppm SO_2 70 ppm SO_2 + 250 ppm CO	7hr/d, days 6 thru 18 of gestation	Rabbits	New Zealand			20 20 20			

Observations:

Maternal Toxicity

Exposure of bred mice and rabbits to 25 or 70 ppm, respectively, of SO_2 alone or in combination with 250 ppm of CO had no significant effect on the dams' appearance, demeanor, body weight gain, or liver weight. Gross and microscopic examination at the time of sacrifice revealed no evidence of toxicity that could be attributed to SO_2 alone or in combination with CO. Incidence of pregnancy was not adversely affected in either species by exposure to SO_2 or SO_2 plus CO.

Embryotoxicity and Fetotoxicity

Exposure of mice to 25ppm SO_2 did not significantly increase the incidence of resorptions of fetuses. Mean fetal body weight was decreased significantly among the litters of mice exposed to SO_2 alone. Exposure of mice to SO_2 in combination with CO produced a greater decrease in fetal body weight than exposure to SO_2 alone, and fetal crown-rump length was reduced significantly among the litters of mice exposed to the combination. In rabbits, exposure to 70ppm did not significantly affect the # of fetuses per litter or the incidence of resorptions. The rabbits exposed to SO_2 plus CO had significantly more resorptions than did the control group or the group exposed to SO_2 alone.

Fetal Alterations

No individual type of malformation occurred at an incidence significantly different from that of the controls. In the mice, delayed ossification of the sternebrae and of the occipital bone were seen significantly more often among the litters of mice exposed to SO_2 alone or in combination with CO than among the controls. In rabbits, several minor skeletal variants occurred significantly more often among the litters of rabbits exposed to SO_2 alone than among the controls.

*Statistically significant

Author's Conclusions:

"SO_2 was not teratogenic in either mice or rabbits inhaling the compound at concentrations which produced mild maternal toxicity."

Review – Assessment:

A. Test animals:	(+)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+)b) Age *Guideline: Adult, i.e.,8-12 weeks.*

	(+)c) Sex (No information) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (+) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(+)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+)Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.*

	(+/-)Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (+) Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? (-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (-)Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?

G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+/-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

In this study, SO_2 was not teratogenic in either mice or rabbits inhaling the compound at concentrations which produced mild maternal toxicity. The authors noted that the few malformations which were observed among the SO_2 exposed litters were types that have been noted to occur spontaneously at a low incidence in previous control groups. There was a significant increase in skeletal malformations in mice and rabbits. The teratogenic potential of SO_2 when inhaled in combination with 250 ppm CO was not significantly different from that of SO_2 alone in either species. The concentrations of SO_2 tested were considerably greater than those that might be encountered by humans either in the environment or the workplace. The results do support human exposure guidelines, however only 2 concentration gradients were used, one for each species. This limits how far the data can be extrapolated to humans.

Confidence index:

This study consisted of a large sample size that gave significant data concerning the teratogenicity of SO_2. The exposure regime was logical and appropriate endpoints were used. Proper control of the experiments was performed to remove confounders due to animal variability. Statistical treatment of the data appeared adequate and for the most part, and variability was not a factor in determining the levels of significance. However, the only comparative study was written by the same authors and there is no description of the monitoring devices. Furthermore, the period of observation after exposure is less than 14 days and the organs of the mice and rabbits were examined differently. In addition, there was only one dose level each for rabbits and mice and there was no information on the previous health status of the mothers.

Review – Confidence index ranking:

Moderate

Author: Mortola JP. Citterio G. Agostoni E.

Internal ID: 141

Title: Sulphur dioxide block of laryngeal receptors in rabbits

Year: 1985

Abstract: In 6 rabbits moving average of activity of superior laryngeal nerve(SLN) increased when pressure in upper airways (Pua) was positive and decreased when it was negative. After SO_2 exposure of upper airways SLN activity at Pua = 0 decreased to 40% and was no longer affected by changes in Pua. Activity of 67 fibers of SLN was recorded in 11 rabbits: 35 came from 'pressure' receptors, 27 from 'drive', and 5 from 'flow'. Thirty-three pressure receptors discharged at Pua = 0: 32 increased their firing rate with positive Pua and decreased it with negative Pua, one did the reverse. One pressure receptor silent at Pua = 0 fired with positive Pua, the other with negative Pua. Pressure receptors were slowly adapting. SO2 blocked within 3-9 min 84% of pressure receptors, 56% of drive receptors, and 4 out of 5 flow receptors. The receptors recovered control activity within 5-10 min after SO_2 removal. SO_2 block of laryngeal receptors may represent a convenient experimental tool for studies of laryngeal reflexes.

Objective: n/a

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
300-350ppm	**10-15 min**	**Rabbit**	**white**	**n/a (1.5-2.0kg)**	**n/a**	**6**	**Presumed healthy**	n/a	n/a

Observations:

"In 6 rabbits the moving average of the activity of the superior laryngeal nerve (SLN) during expiration increased when the pressure across the upper airways was positive and decreased when it was negative…After exposure to SO_2 the activity of the SLN was reduced to about 40% of control, i.e., to a value similar to that obtained with a transmural pressure (Ptm) of -20cm H_2O before SO_2. After SO_2, the activity of the SLN was no longer significantly affected by changes in Ptm from -20 to 10cm H_2O."

*Statistically significant

Author's Conclusions:

"In conclusion, in rabbits we found laryngeal receptors which correspond to the pressure, drive and flow receptors observed…in dogs. Most of the pressure and, possibly, flow receptors and about one half of the drive receptors are blocked by SO_2."

Review – Assessment:

A. Test animals:	+✏ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* +✏ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex -d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✏ Were the chosen exposure levels appropriate to investigate the primary objective? -✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? ±✏ Was the duration of exposure precisely defined? +/-✏ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? +✏ Was evidence provided to justify the dosing regime? -✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	-✏ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✏ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +/-✏ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.*

	-✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? -✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? -✎ Is the control regime appropriate? -✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? -✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? -✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	-✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? -✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?

H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? -✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study determined that laryngeal receptors similar to those of dogs exist in rabbits. Most of the pressure and flow receptors and approximately half of the drive receptors appeared to have been blocked by SO_2. Hence changes in timing and diaphragm activity previously observed after exposure to SO_2 of the upper airways in rabbits might be due to removal of laryngeal inputs. While these results seem significant, the author also speculates that changes could depend on effects of SO_2 on receptors or regions rostral to the larynx. Without including a control group into the study, correlating removal of laryngeal inputs with diaphragm activity is not possible. It therefore may be reasonable to assume that the behavior of the receptors in humans is also different.

Confidence index:

The results in this study are not very informative due to many shortcomings in the study design. No control group was assayed and only 6 rabbits were included in the first experiment and 11 in the second. There is no information regarding characteristics of the rabbits. A gradient of exposure would be more meaningful to determine if the blockage of the receptors is dose related. The dosage is very high and no justification is given for this dosage level. If this study is to be extrapolated to humans, which is not stated, then the dosage level is far out of proportion to any plausible human exposure. The objective of this study was reached, but the application to humans is not clear. The rabbits were anesthetized and the SO_2 was delivered through a Teflon tube directly connected to the laryngeal cannula. Justification for this dosing route is not given. It is clear that SO_2 appears to block laryngeal receptors in these rabbits, but the 'bigger picture' is not apparent, i.e. Why is this important.

Review – Confidence index ranking:

Low to moderate

Author: Matsumura, Y.

Internal ID: 142

Title: The Effects of Ozone, Nitrogen Dioxide, and Sulfur Dioxide on the Experimentally Induced Allergic Respiratory Disorder in Guinea Pigs 1. The effect on Sensitization with Albumin through the Airway

Year:1970

Abstract: The effects of pre-exposure to ozone, nitrogen dioxide, and sulfur dioxide at various concentrations on sensitization with albumin through the respiratory airways in guinea pigs were investigated. Egg albumin and bovine serum albumin were used as the sensitizing antigens. The degrees of sensitization in the animals pre-exposed to a gas and the control animals were compared by the prevalence of anaphylactic attack after inhalation of antigens, by active cutaneous anaphylaxis tests, and by serologic tests, such as the indirect hemagglutination test. Repeat 30-minute preexposure to more than 5ppm ozone, 70ppm of nitrogen dioxide, and 330 ppm of sulfur dioxide enhanced sensitization via the airways in guinea pigs; One ppm of ozone, 40 ppm of nitrogen dioxide and 180 ppm of sulfur dioxide, however, showed no such effects. Repeated pre-exposure to 10 ppm of ozone did not affect antibody formation against intraperitoneally introduced albumins.

Objective: To investigate the effects O_3, NO_2, and SO_2 at various concentrations might have on sensitization of guinea pigs through the airway.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
20 ppm 60 ppm 180 ppm 330 ppm	30 minutes	Guinea pigs	No info	No info	female	12-18 per exposure group	No info	N/A	N/A

Observations:

General observations on the behaviour of animals exposed to gases:

Only when exposed to 330 ppm of SO_2, the animals became uneasy, sneezed and rubbed their eyes or noses with their paws immediately after initiation of the exposure. These signs of irritation subsided within a few minutes. No significant respiratory distress developed in any animal.

Serologic Analyses:

Five serum samples of 10 guinea pigs exposed to 330 ppm of SO_2 showed hemagglutination, whereas serum from only 3 and none from the control animals showed a reaction. The differences were significant at the 5 per cent level by the Fisher test.

Active cutaneous anaphylaxis test:

The mean of minimal antigen doses required to cause a reaction was much smaller in the animals exposed to 70 ppm NO_2 and those exposed to 330 ppm of SO_2 than in the respective control animals. These differences were significant at the 5 percent level by the t test.

*Statistically significant

Author's Conclusions:

"From these results it can be concluded that repeated, 30 minute pre-exposures to 330 ppm of sulfur dioxide enhanced sensitization with albumin via the respiratory airway in guinea pigs."

Review – Assessment:

A. Test animals:	(+)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (-)c) Sex (No information) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (-) (No information given) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(No)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+). Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (-)Was the duration of exposure appropriate to investigate the primary objective? (-)Was the duration of exposure precisely defined?

	(+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+)Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-)Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
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E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? (-) Were test animals randomly assigned to exposure groups?

	(-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
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G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially

	bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Guinea pigs were exposed to an increasing gradient of concentrations of SO_2 (10, 60, 180, 330 ppm) to determine the effects of pre-exposure to SO_2 on allergic sensitization. The only level of SO_2 that produced any effects was 330 ppm SO_2. This level of SO_2 enhanced the sensitization via the airways in guinea pigs. There were no effects at 180 ppm of SO_2. The results of the two lower levels were not given. The mechanism of the enhancement of sensitization by the pre-exposure to gases has not been clarified in this study.

Confidence index:

This study tried to limit some confounding variables by using same weight animals. The sex and age of animals was not specified though and this is a consideration especially when only half of the animals showed a response. The experimental design was sound and sufficient details are given so that methods should be easily reproducible. Conclusions are in agreement with other published data. Furthermore, there were two exposure chambers, each with different dimensions that were used. In addition, there was no information on the methods of blood analyses.

In some experiments, the serial dilutions of egg albumin were not sufficient to determine the minimal threshold dose in every animal, and the difference between each gas-exposed group and control group could not be distinguished. Finally, the inhalation of antigen aerosols were repeated five to seven times at intervals of a few days for some animals and in some experiments it was repeated every day.

Review – Confidence index ranking:

Low to moderate

Author: Matsumura Y	**Internal ID: 143**
Title: The effects of ozone, nitrogen dioxide, and sulfur dioxide on the experimentally induced allergic respiratory disorder in guinea pigs. 3. The effect on the occurrence of dyspneic attacks.	
Year: 1970	
Abstract: The effect of pre-exposure to ozone, nitrogen dioxide, and sulfur dioxide on the occurrence of anaphylactic dyspnea experimentally provoked by antigen inhalation in guinea pigs was investigated. A mixture of egg albumin and bovine serum albumin was used as sensitizing antigen. The antigen solution was injected intraperitoneally so all animals would be sensitized equally and would later have only a slight anaphylactic attack after inhalation of antigen aerosols. Immediately after the inhalation of antigen, almost all animals pre-exposed to more than 2ppm ozone and more than 40ppm of nitrogen dioxide had severe dyspneic attacks, and many of them died, whereas most of the control animals showed only slightly labored breathing. Pre-exposure to sulfur dioxide at a concentration of 400ppm, however, did not enhance the occurrence of the paroxysm of anaphylactic dyspnea.	
Objective: "The purpose of the present study was to investigate what effect O_3, NO_2, and SO_2 might have on the occurrence of paroxysms of dyspnea provoked in guinea pigs by the inhalation of antigen aerosols."	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
400ppm	**30min**	**Guinea pig**	**n/a**	**300grams**	**female**	**62**	**Healthy**	**n/a**	**n/a**

Observations:

"…signs of irritation were observed only in animals exposed to SO_2 at 400ppm; these signs occurred immediately after initiation of exposure and subsided in a few minutes. No significant disturbance of respiration was seen in any case…"
While many animals died after exposure to other gases "… the animals exposed to SO_2, however, showed only slightly labored breathing as did the control animals."

*Statistically significant

Author's Conclusions:

"The exposure to SO_2 however, at a concentration as high as 400ppm did not show the effect seen in the experiments with O_3 and NO_2. This evidence suggests some of the mechanisms of the effect of O_3 and NO_2… It is generally known that SO_2 is much more soluble in water and penetrates less deeply into the respiratory system than O_3 and NO_2."

Review – Assessment:

A. Test animals:	+✏ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✏ Are animal characteristics adequately recorded and appropriate for the objective of the study?

	+a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult. i.e.,8-12 weeks.* +c) Sex -d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? +✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water*

	may be withheld optionally.
D. Equipment:	-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* -✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? -✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	-✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? ±✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? ±✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? ±✎ Did either the study design or the statistical analysis limit confounding variables? ±✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable?

	+✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:
31 guinea pigs were exposed to one concentration of sulfur dioxide (400 ppm) in order to determine what effect this would have on an already induced allergic response. This study does not support human exposure guidelines because only one concentration was used in an acute manner. While the results are interesting in comparison to ozone and nitrogen dioxide, this study has little value for the present purpose.

Confidence index:
The study methods are very poorly described, and statistics were not used to analyze the data. Furthermore, the length and type of assessment does not appear to be thorough. The study tried to limit confounding variables by using same sex, same weight guinea pigs. The age was not recorded though and it is hard to say whether the conclusions can be extrapolated for both adults and young. For this study only a single endpoint was chosen to monitor, paroxysms of anaphylactic dysnea. Perhaps if additional parameters (ie respiratory flow resistance etc) were considered more information could have been gained regarding effects of SO$_2$ exposure. Confidence in the results of this study is expected to be relatively low, despite the large sample size (n=62).

Review – Confidence index ranking:

Low to moderate

Author: Matsumura, Y., Mizuno, K., Miyamoto, T., Suzuki, T., Oshima, Y. **Internal ID:** 144

Title: The Effects of Ozone, Nitrogen Dioxide, and Sulfur Dioxide on the Experimentally Induced Allergic Respiratory Disorder in Guinea Pigs IV. Effects on Respiratory Sensitivity to Inhaled Acetylcholine

Year: 1972

Abstract: The effects of pre-exposure to ozone, nitrogen dioxide and sulfur dioxide on the sensitivity of guinea pigs to inhaled acetylcholine were investigated. Immediately after inhalation of nebulized acetylcholine was initiated, almost all animals pre-exposed to more than 2 ppm of ozone and more than 50 ppm of nitrogen dioxide manifested severe difficulty n respiration similar to anaphylactic attacks, and many of them died whereas most of the control animals showed labored breathing but did not die. There were, however, no differences in respiratory signs between the group of pre-exposed to sulfur dioxide at a concentration of 450ppm to 700 ppm and the control group. The effect of ozone persisted for 2 hours after the exposure and was not evident after 5 hours, but appeared again after 23 hours. Pre-treatment with ozone at 1ppm blocked the effect of ozone at more than 2ppm.

Objective: The purpose of the present study was to investigate the effect of O_3, NO_2, and SO_2 on the respiratory sensitivity of guinea pigs to inhaled acetylcholine, one of the chemical mediators.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
1.450ppm 2. 600 ppm 3. 700 ppm	30 minutes	Guinea Pigs	No info	No info	Male	1. 8 (8 control) 2. 8 (8 control) 3. 13 (13 control)	No Information	unknown	unknown

Observations:

Immediately after inhalation of nebulized acetylcholine was initiated, almost all animals pre-exposed to more than 2 ppm ozone and more than 50 ppm of nitrogen dioxide manifested severe difficulty in respiration similar to anaphylactic attacks, and many of them died whereas most of the control animals showed labored breathing but did not die. There were, however, no differences in respiratory signs between the group pre-exposed to sulfur dioxide at a concentration of 450 ppm to 700 ppm and the control group. The effect of ozone persisted for 2 hours after the exposure and was not evident after 5 hours, but appeared again after 23 hours. Pre-treatment with ozone at 1 ppm blocked the effect of ozone at more than 2 ppm.

*Statistically significant

Author's Conclusions:

There was no difference in respiratory signs between the group pre-exposed to sulfur dioxide at any concentration and the control group. Exposure to SO_2 even at

concentrations more than 450 ppm did not produce the effects of O_3 and NO_2.

Review – Assessment:

A. Test animals:	(+)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+)c) Sex (No information) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (-) (No information given) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+/-)Was the mode of administration appropriate?

	(+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (-)Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-)Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-)Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? (+) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate?

	(-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(-) Were appropriate endpoints examined? (-)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+)Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The motivation behind the study was that if pollutants such as SO_2 change the respiratory sensitivity to chemical mediators, certain environments might induce asthmatic attacks in persons who do not otherwise suffer from asthma. In this study, using guinea pigs, there were no differences in respiratory signs between the group pre-exposed to sulfur dioxide at a concentration of 450-700 ppm. In addition, there was no difference in respiratory signs between the group pre-exposed to sulfur dioxide at any concentration and the control group.

Confidence index:

The observations in this study are in agreement with previously published data with the exception that one group noticed an inhibition of acetylcholinesterase activity in mice exposed to 10 ppm. However, this study is with a different species and inhibition of acetylcholinesterase doesn't necessarily mean increased sensitivity to acetylcholine would occur. Study tried to limit confounding variables by using same sex animals, but didn't record age or pre-trial health. Pre, actual and post exposure conditions were not well defined. For this study only a single endpoint was chosen to record, mortality. However, there was no justification for the high exposures levels. Perhaps if additional parameters (ie respiratory flow resistance etc) were considered more information could have been gained regarding effects of SO_2 exposure. Furthermore, the interval between SO_2 exposure and acetylcholine varied. For 700 and 600ppm, it was 45 minutes. For 450ppm it was 10 minutes.

Review – Confidence index ranking:

Low to moderate

Author: Xu J. Zhao M. Liao S.

Internal ID: 145

Title: Establishment and pathological study of models of chronic obstructive pulmonary disease by SO_2 inhalation method.

Year: 2000

Abstract: OBJECTIVE: To establish rat models of chronic obstructive pulmonary disease (COPD) and study the pathological characteristics of airflow obstruction. **METHOD:** SO_2 inhalation method was used to establish rat models. After exposure to SO_2 for 7 weeks, peak expiratory flow (PEF), peak inspiratory flow (PIF), intratracheal pressure (IP) and IP slope in rat were measured by Maclab data recording and analysis system. Experimental rats with PEF less than 80% of the mean of the normal rats were classified as airflow obstructed, while those with PEF greater than 80% of mean of normal rats were non-obstructed. Pathological changes in airway and lung tissue were compared between these two groups. **RESULT:** In experimental animals, PEF was significantly decreased ($P < 0.005$) and IP slope increased ($P < 0.001$) as compared with normal rats. Epithelial damage, goblet cell hyperplasia and inflammatory cell infiltration in cartilaginous bronchi were more remarkable in experimental rats with airflow obstruction than those without airflow obstruction ($P < 0.001$, < 0.01, < 0.001, respectively). Furthermore, pathological changes in airway lumen, epithelium and airway wall in membranous and respiratory bronchioles were more marked in experimental rats with airflow obstruction than those without airflow obstruction ($P < 0.001$ or $P < 0.05$). There was a negative correlation between PEF values and epithelial hyperplasia, goblet cell hyperplasia, inflammatory cell infiltration, smooth muscle hyperplasia and mucous plug in membranous and respiratory bronchioles ($P < 0.001$ or $P < 0.05$). **CONCLUSION:** SO_2 inhalation may cause chronic bronchitis with airflow obstruction, i.e. COPD in rats. COPD was induced in 64% (16 of 25) of the experimental group rats.

Objective: "To establish rat models of chronic obstructive pulmonary disease (COPD) and study the pathological characteristics of airflow obstruction."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
250ppm	**5h/day 5d/week for 7 weeks**	**rat**	**wistar**	**4 weeks**	**male**	**45**	**Healthy**	**n/a**	**n/a**

Observations:

Researchers noticed increased deaths, decreased PEF ($p<0.005$) and increased IP slope ($p<0.001$) in SO_2 exposed rats relative to the control. 16 of 25 rats in the SO_2 exposed group developed what can be considered COPD, while the remaining 9 had characteristics of chronic bronchitis. Furthermore, other signs of pathology and inflammation were increased in the SO_2-exposed group.

*Statistically significant

Author's Conclusions:

"Our data show that airflow obstruction existed in 16 of 25 experimental (SO_2-exposed) animals (64%). We also show that SO_2 inhalation may cause pathologic change typical of chronic bronchitis with airflow obstruction, i.e., COPD. COPD in this rat model was supported by measurements of airflow obstruction such as PEF."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* +b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex +d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	-*✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.*

	-✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* -✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? -✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? +✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? ±✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? ±✎ Was the design of the study appropriate to investigate the primary objective?

	+✎ Were assumptions reasonable? +✎ Are the methods reproducible? -✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings demonstrate that high level exposures (250ppm) for a significant period of time (~1week) can induce disease similar to chronic bronchitis and COPD in a rat model. Thus, SO_2 exposure can produce long-lasting effects, in addition to the transient effects often reported. The purpose of the study was not to determine what dose/exposure of SO_2 initiated this disease, rather the goal was to initiate the disease and determine what the rat model of the physiology would be. Therefore, this study does not offer support to human exposure guidelines other than to support the fact that chronic exposure to high levels of SO_2 will permanently damage the respiratory tract.

Confidence index:

This study used a well defined existing protocol to induce chronic bronchitis in rats. They then studied airflow obstruction extensively by measuring multiple parameters. Their data document pathological changes typical of COPD. Airflow obstruction occurred in 16 of 25 experimental animals, however, the sample size was small and results warrant further studies.

Review – Confidence index ranking:

Moderate

Author: Norris, A.A., Jackson, D.M.	**Internal ID:** 146
Title: Sulphur dioxide induced airway hyperreactivity and pulmonary inflammation in dogs	
Year:1989	
Abstract: Anaesthetized Beagle dogs were exposed to sulphur-dioxide (SO_2) for 2 h and the pulmonary effects studied up to 24 h using bronchoalveolar lavage (BAL) and lung mechanics measurements. SO_2-induced hyperreactivity to histamine was associated with epithelial cell shedding, an increase in airway permeability to plasma proteins and an increase in BAL PGE2 content. Leukotrienes were not recovered in BAL. Ibuprofen reduced hyperreactivity at 24-h post SO_2 but not immediately after SO_2 and had no effect on BAL cells at anytime. These results suggest that 2 phases of hyperreactivity exist--an immediate effect which may involve epithelial cell loss and a later phase which may be inflammatory.	
Objective: To determine the cellular, permeability and inflammatory mediator changes in the lungs of dogs exposed to SO_2 and investigate the correlation between these changes and the increased lung reactivity to histamine aerosols. To investigate the effects of the cyclooxygenase inhibitor, ibuprofen, on the changes induced by SO_2.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
200 ppm	2 hours	Dogs	Beagle	Adult	Male and female	7	No info	N/A	N/A

Observations:

Airway hyperreactivity was observed in all dogs exposed to SO_2. An increase in responsiveness to aerosolized histamine following SO_2 occurred in all dogs. This was associated with epithelial cell shedding, an increase in airway permeability to plasma proteins and an increase in bronchoalveolar lavage PGE2. Ibuprofen pretreatment produced a delay effect on airway responsiveness to histamine by reducing significantly the reactivity at 24 h post SO_2.
*Statistically significant

Author's Conclusions:

"In conclusion, we have shown that a model of airway hyperreactivity in dogs induced by SO_2 is associated with significant inflammatory changes within the lung. Results with ibuprofen suggest it is more likely that inflammation is responsible for hyperreactivity in the later phase (24h) than immediately after SO_2."

Review – Assessment:

A. Test animals:	(+/-)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.*

	Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation *Guideline: Below 20% variation* (-) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content

	maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+/-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? (-) Were test animals randomly assigned to exposure groups? (+/-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random Assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) appropriate endpoints examined? (+)Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed?

	(+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

An unspecified number of dogs were exposed to 200 ppm in order to determine the pulmonary effects. This study used only one concentration gradient, and did not offer any justification of why dogs were used, or even this breed of dog. These factors, coupled with other flaws in the study render it valueless to human exposure guidelines.

Confidence index:

Test animals were beagles – there was no justification for their use. Also, there is no evidence that researchers tried to limit confounding variables - no record of exact number of animals, pre-trial health, their source or their sex. No justification for exposure concentration (200 ppm only) or duration. The administration of ibuprofen occurred both before and after exposure to SO_2 making it very difficult to interpret the data. Although their interpretation that ibuprofen inhibits the response to histamine following SO_2 exposure by preventing inflammation induced reactivity is a reasonable extrapolation the mechanism needs to be clarified. Also, their conclusions with ibuprofen are derived from comparison of cell numbers of neutrophils, epithelials or macrophages following SO_2 exposure to untreated controls – these numbers are significantly different. However, if you look at cell numbers following SO_2 exposure between ibuprofen pretreated and not pretreated there is appears to be no difference.

Also, the exposure assessment was questionable. Dogs were sedated and then anaesthetized. There was no information on the possible effects of these drugs.

Review – Confidence index ranking:

Low

DRAFT

Author: Rana SV. Gautam RK. Agrawal VP	**Internal ID:** 147
Title: Certain biochemical changes in the trachea, lungs, and heart of squirrels exposed to three principal air pollutants.	
Year: 1979	
Abstract: The fate of total lipids, proteins and carbohydrates in the trachea, lungs, and heart of the common ground squirrel, Funambulus pennanti, have been determined after separate exposure to three principal air pollutants, carbon monoxide (CO), sulphur dioxide (SO_2), and nitrogen dioxide (NO_2). Exposure to all of these gases produced edematous conditions; however, the moisture content of heart muscle was reduced. Carbon monoxide (CO) and NO_2 are more toxic to pulmonary lipids than SO_2. The lipid of the heart decreased, least effects noted with SO2 treatment. Mechanical properties of the lungs were changed by alteration of the lung lipids causing changes in the surface tension. Changes in protein content were caused by altered membrane permeability. Comparative data on the carbohydrates indicated adverse effects by the pollutants.	
Objective: "This investigation focuses attention on the fate of total lipids, proteins, and carbohydrates in the trachea, lungs, and heart of the common ground squirrel, *Funambulus pennanti*, when it is exposed to high concentrations of the three gases separately."	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
500ppm	4min	Common Ground squirrel		n/a 90grams	n/a	5	Healthy	n/a	n/a

Observations:

Squirrels exposed to pollutant gases gained more water than control squirrels. SO_2 decreased the amount of moisture in the trachea and heart muscles. Exposure to SO2 was damaging to lipid fraction and protein, though not to the extent of CO exposure. Carbohydrate contents of lung and heart tissues were diminished.
*Statistically significant

Author's Conclusions:

"…this study indicates that NO does induce edema. CO and SO2 are also edematous… A decrease in the moisture content of cardiac muscle poses a serious problem, and it would be important to know what is responsible for this loss of moisture."
"The lipid data show that CO and NO_2 are much more toxic to pulmonary lipids than is SO_2."
"The relationship between tissue proteins, SO_2 and CO inhalation deserves special attention. The interaction of SO_2 with tissue proteins may be due to its reaction with water to form sulfite and bisulfite anions, and these ions further react with disulfide bond in a receptor protein."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie

	☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex -d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? -✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	-✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* +✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*

D. Equipment:	-✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* -✏ Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✏ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? -✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? -✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✏ Was individual data reported for each test animal?
G. Data analysis:	-✏ Are the statistical methods used appropriate? -✏ Are confidence intervals reported? +/-✏ Did the investigator appropriately interpret the statistics? -✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? -✏ Was the design of the study appropriate to investigate the primary objective? +✏ Were assumptions reasonable? -✏ Are the methods reproducible? -✏ Did either the study design or the statistical analysis limit confounding variables? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal?

	+✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:
Ground squirrels were used to determine the biochemical changes in the trachea, lungs and heart after exposure to SO_2, CO, NO_2 for 4 minutes at 500 ppm four minute exposure. No justification was given why this species was chosen or what similarities, if any, it bears to humans. Therefore, this study has no value to human exposure guidelines.

Confidence index:
The methods section is very rudimentary in nature and does not provide sufficient descriptions of either study animals or exposure procedures. Study design allotted 5 animals to each treatment, and to a control, though sex and age were not specified. The increase in fluid weight of the lung following acute SO_2 exposure is curious given that the decrease in moisture content of the trachea was attributed to the hygroscopic nature of SO_2 and the total protein, lipid and carbohydrate content of the lungs decreased significantly under these conditions. Furthermore, no details of statistical analyses were not provided. As such, this study has limited confidence.

Review – Confidence index ranking:

Low

Author: Majima Y. Swift DL. Bang BG. Bang FB	**Internal ID: 149**

Title: Mechanism of slowing of mucociliary transport induced by SO_2 exposure.

Year: 1985

Abstract: Responses of nasal mucociliary transport mechanisms to exposure to 6 ppm SO_2 were studied in chickens in vivo. This model takes advantage of the natural cleft palate which exposes the mucociliated base of the nasal septum. Exposure to 6 ppm SO_2 decreased the mucociliary transport rate along the base of the nasal septum. The minimum force required to move an iron particle along this area of mucous membrane by use of a magnetic field in vivo increased significantly after SO_2 exposure, while the minimum force required to move an iron particle on a pool of mucus collected from the same chicken and tested in vitro showed no change after SO_2 exposure. The elastic recoil distance of mucus was measured both in vivo and in vitro. The in vivo recoil distance decreased significantly after SO_2 exposure, while SO_2 exposure did not change recoil distance in vitro. It is proposed that exposure of chickens to SO_2 results in the formation of multiple points of adhesion of strands of mucus between the acinar gland cells and the emergent extracellular mucus or adhesion of a mucous blanket to the cilia, causing mucociliary transport to be retarded or static.

Objective: "In this paper, we propose the hypothesis that slowing of mucociliary clearance is brought about by the tethering of emergent mucus to residual mucus within the goblet cells and acini."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
6ppm	16h/day for 7 days	chicken	White leghorn	19-29days	n/a	12	Healthy	n/a	6ppm

Observations:

"Mucociliary transport was absent in 55 instances (44%) of the SO_2 group and in 7 (5%) of the control group. This difference was significant at $p<0.005$. This complete cessation of transport increased in frequency with time after SO_2 exposure, reached a maximal value on the third day, returned toward normal on the fifth day of the exposure, and then again increased."

"…the mean value of Fviv (minimum force in vivo – a measure of viscosity of mucus) in chickens exposed to SO_2 was significantly elevated with the onset of exposure, and was maximal on the third day of exposure (0.200±0.032 dyne). This value was significantly different than both the mean control value (0.076±0.018 dyne) and the value before SO_2 exposure (0.083±0.027 dyne) ($p<0.05$)."

At no time was there a difference in Fvit (minimum force in vitro) among controls and SO_2 treated animals.

Recoil distance decreased significantly in vivo following exposure to SO_2 ($p<0.005$), but not in vitro.

*Statistically significant

Author's Conclusions:

"Recent reports suggest the important effect of the viscoelastic property of mucus on mucociliary transport. Does disturbance of this property of mucus play a role in the slowing of mucociliary transport by SO_2? Our failure to find any correlation between SO_2-induced slowing and change in in vitro elastic properties of mucus is not consistent with this explanation, because during acute SO_2 exposure, no change of recoil distance in vitro and Fvit in collected mucus was observed. We believe that the slowing of mucociliary transport during SO_2 exposure is best explained by an in vivo physical factor, such as tethering, which requires greater force to move the surface layer of mucus."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? -a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex -d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* +f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? +/-✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +/-✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%*

	-✏ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✏ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* +✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* +✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✏ Was a full description of the monitoring devices provided? +/-✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✏ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? -✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? ±✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? -✏ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? ±✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? -✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? -✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✏ Was individual data reported for each test animal?
G. Data analysis:	+✏ Are the statistical methods used appropriate? +✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics?

	+✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective? +✏ Were assumptions reasonable? +✏ Are the methods reproducible? -✏ Did either the study design or the statistical analysis limit confounding variables? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings allow for the development of a hypothesis suggesting that increased mucus viscosity and decrease mucus flow rates following SO_2 exposure are the result of an in vivo physical factor, rather than the viscoelastic properties of the mucus. These findings, if they can be applied to humans, may have implications for the development of treatments for persons adversely affected by SO_2 exposures.

Confidence index:

The methods section refers the bulk of the content to another paper, so it is difficult to judge the quality of the methods used to expose the birds. This paper tries to identify possible mechanisms for the decreased mucociliary clearance rate following SO_2 exposure (a well established effect of SO_2 in man and animals). There is no justification for the use of chickens for this study. The exact exposure conditions were not defined, however there was a full description of assessment methods making them easily reproducible. Several models are proposed to explain the decreased transport rate – all involve an in vivo factor such as tethering of mucus between acinar gland cells or adhesion of mucus to the cilia but no histological evidence is provided to support these mechanisms.

Review – Confidence index ranking:

Low to moderate

Author: Man, S., Hulbert, W., Mok, K., Ryan, T., Thomson, A	**Internal ID:**150
Title: effects of sulfur dioxide on pore populations of canine tracheal epithelium.	
Year: 1986	
Abstract: The bioelectric and barrier properties of the tracheal epithelium in nose-breathing dogs and in dogs that had been exposed for 75 min to compressed air or to two high concentrations of SO_2 were measured and compared. We also studied tissues that had been treated with chloroform. Based on a model of restrictive diffusion we demonstrated heteropores (6 and 250 A) in the control tissues. Bioelectric changes due to 100 ppm SO_2 were minimal. After exposure to 500 ppm SO_2, adverse changes in the bioelectric properties were focal; they were marked in 8 out of 12 animals but were less striking in the other 4. Nonelectrolyte permeability increased with an increase in SO_2 concentrations. Small pores were still present in the tissues severely affected by SO_2 but they were absent in chloroform-treated tissues. Scanning electron microscopy of tissues from animals exposed to 500 ppm SO_2 showed that in the same dog tissue appearance varied from normal to one of repair (normal bioelectric properties) or one of marked exfoliation of ciliated cells (abnormal bioelectric measurements).	
Objective: This study was conducted first to define the pore populations in the airway mucosa, second to document the acute effects of SO_2 at high concentrations on the bioelectric properties and the pore populations, and tried to assess the correlation between surface morphology and measurements of the pore populations and bioelectric properties.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
100, 500 ppm	75 minutes/once	dogs	mongrel	NA	Both	35	Healthy	No observed adverse effect level	Lowest observed adverse effect level

Observations:

In addition to the two exposure groups above, there was Group A (control, n=6), Group B (compressed air, n=6) and Group E (n=3, the tracheal epithelium was treated with chloroform to digest the cell membrane.) In the 500ppm SO_2 group (group D), the effects were variable among dogs as well as in the same dog. It is clear that profound damage had occurred in the majority of the tissues but 21 out of 86 had reasonably good bioelectric properties. Some dogs had normal PD (transepithelial potentials), I_{sc}(short circuit current), or R (resistance) to suggest healthy tissues; others had grossly altered bioelectric measurement to suggest severe damage. After exposure to 500ppm SO_2, adverse changes in the bioelectric properties were focal; they were marked in 8 out of 12 animals. Nonelectrolyte permeability increased with an increase in SO_2 concentration. Small pores were still present in the tissues severely affect by SO_2 but they were absent in chloroform-treated tissues. The morphological effects of 500 ppm were variable.
*Statistically significant

Author's Conclusions:

"500 ppm SO_2 exposure lead to variable and focal distribution of epithelial damage along the trachea. Results are consistent with the notion that the large nonciliates that were seen following SO_2 injury may provide excellent barrier function."

Review – Assessment:

A. Test animals:	(-) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (-) c) Sex (+) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (-) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(+) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the*

	chamber equilibrates. (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random

	assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Dogs were exposed to two concentrations of SO_2 in order to determine what changes occurred in the pore structure and bioelectric properties of the tracheal epithelium. High concentrations of SO_2 had an adverse effect on the bioelectric properties of the tracheal epithelium in dogs, the variable and focal distribution of epithelial damage along the trachea are consistent with the notion that the large nonciliates that were seen following SO_2 injury may provide excellent barrier function. The findings in this study are unreliable due to the large variability in responses and problems with the study design. Exposures to 500ppm are also not likely to occur in the general population.

Confidence index:

The study design did attempt to limit confounding factors by using controls both for exposure and also for experimental parameters (intubation and respiratory circuitry), however, it did not define the sex, weight or age of the animals. Furthermore, there is no justification for using dogs and there is greater than a 20% weight variation.. In addition, there was inter- and intra- individual variability. Four example, 4 out of the 12 dogs in the 500 ppm group had normal bioelectric measurements in at least half of the tissue chambers, 5 dogs had very poor PD and 3 dogs had a couple of chambers with seemingly normal bioelectric properties among the ones with poor PD.

This study complemented abnormal permeability data with morphological data demonstrating that SO_2 exposure led to boiling exfoliation of ciliated cells. Findings are in agreement with other published works in other animals suggesting effects of SO_2 can be extrapolated

Review – Confidence index ranking:

Moderate

Author: Jonek J. Konecki J. KoSmider S. Kaminski M.	**Internal ID: 151**
Title: The effect of $^{35}SO_2$ binding by ammonia on sulfur incorporation into rat tissues.	
Year: 1976	
Abstract: Sulfur metabolism was traced in experimental investigations on rats, and the importance of the method of sulfur dioxide neutralized by ammonia was estimated. The animals were exposed to $^{35}SO_2$ by means of inhalation, and radioactivity in several organs at different periods of exposure was traced. Apart from that, autoradiographic investigations were carried out in order to show the incorporation of radioactive sulfur into particular elements of the cell in different tissues. Elimination of isotope with urine and feces at different periods of time after exposure was also determined. It was found that the use of ammonia to neutralize sulfur dioxide in the air decreased the amount of sulfur which could enter the organism as a result of breathing air contaminated by SO_2.	
Objective: "The purpose of this work was to trace ^{35}S metabolism in rats exposed to $^{35}SO_2$ with simultaneous application of the method of sulfur dioxide binding by ammonia."	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
2.5-2.8µg/C	**50min single exposure**	**rat**	**Wistar**	**n/a**	**male**	**52**	**n/a**	**n/a**	**n/a**

Observations:

"In rats exposed to inhalation of the air contaminated by $^{35}SO_2$, sulfur enters the blood and tissues. Two hours after exposure to $^{35}SO_2$ the highest radioactivity was found in the serum and then in the liver, kidneys, lungs, heart, testicles and brain. It was found that radioactivity was still high in the serum, and from among the organs investigated, in the kidneys and lungs 24hrs after the exposure. On the following days radioactivity in the serum was decreased, while the kidneys and lungs it was kept at a high level. After binding $^{35}SO_2$ contained in the air by ammonia, the amount of ^{35}S in the serum and the investigated organs."
*Statistically significant

Author's Conclusions:

"These results obtained indicate that a considerable radioactivity in serum, lungs and kidneys of the rats occurs only 2hrs after the exposure to the air contaminated by $^{35}SO_2$. These data suggest that sulfur contained in SO_2 passes quickly from lungs into the blood, and is then eliminated with urine, mainly during the first day. Some parts of the absorbed sulfur are eliminated with feces."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie

	☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ±✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? -✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	-✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* ±✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*

D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* -✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? ±✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? ±✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal?

	+✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings demonstrate that, at the very least, S contained in SO_2 is absorbed by the tissues of rats in relatively large quantities, even shortly after exposure (2hr). These findings could have implications for effects of SO_2 exposure beyond the respiratory tracts.

Confidence index:

The ability of ammonia to neutralize the effect of SO_2 was demonstrated by a decrease in the amount of radioactive ^{35}S in multiple tissues. This study also identifies the organs in which SO_2 accumulates and is likely metabolized in. Experimental details for both exposure and assessment are well defined and should be easily reproducible. Iinformation deficiencies as to animal health, diet, reagent source, monitoring equipment sensitivity, assignment to test groups, actual numbers of animals exposed to concentrations lower confidence in this study.

Review – Confidence index ranking:

Moderate

DRAFT

Author: Lovati, M.R., Manzoni, C., Daldossi, M., Spolti, S., Sirtori, C.R.

Internal ID: 152

Title: Effects of sub-chronic exposure to SO_2 on lipid and carbohydrate metabolism in rats.

Year: 1996

Abstract: Sulfur dioxide (SO_2) is a ubiquitous air pollutant, present in low concentrations in the urban air, and in higher concentrations in the working environment. While toxicological reports on SO_2 have extensively dealt with the pulmonary system, essentially no data are available on the effects of chronic exposure to this pollutant on intermediary metabolism, although some biochemical changes in lipid metabolism have been detected. The present investigation was aimed at evaluating the effects of sub-chronic exposure to SO_2 on concentrations of serum lipids/lipoproteins and on glucose metabolism, in animal models of hypercholesterolemia and diabetes. A specially designed control-inert atmosphere chamber was used, where male Sprague-Dawley rats fed on either standard or cholesterol enriched (HC) diets, as well as streptozotocin diabetics, were exposed to SO_2 at 5 and 10 ppm, 24 h per day for 14 days. In rats, both on a standard diet and on a HC regimen, SO_2 exposure determined a significant dose-dependent increase in plasma triglycerides, up to +363% in the 10 ppm HC exposed animals. This same gas concentration significantly reduced HDL cholesterol levels. In contrast, exposure of diabetic animals to 10 ppm SO_2 resulted in a fall (-41%) of plasma and liver triglycerides and in a concomitant increase (+62%) of plasma HDL cholesterol. This discrepancy could apparently be related to diverging effects of SO_2 exposure on plasma insulin levels in the different animal groups. Kinetic analyses of triglyceride synthesis carried out in rats on a standard diet revealed, in exposed animals, a significant reduction in the secretory rate, in spite of the concomitant hypertriglyceridemia. These findings suggest that SO_2 exposure can markedly modify major lipid and glycemic indices, also indicating a differential response in normo/hyperlipidemic versus diabetic animals.

Objective: To evaluate the effects of sub-chronic exposure to SO_2 on concentrations of serum lipids/lipoproteins and on glucose metabolism, in animal models of hypercholesterolemia and diabetes.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
5 ppm and 10 ppm	24 hrs/d for 15 d	Rats	Sprague-Dawley CD	No info	male	81 total (Divided into 3 groups. Within each group, 3 subgroups were formed, of nine animals each) Equal numbers of control	Unknown	N/A	N/A

						groups			

Observations:

Effect of SO_2 exposure in rats fed a standard diet
The body weight gain was reduced in rats fed a standard diet and exposed to SO_2, compared to respective controls. In these animals an increasing fed consumption, reaching statistical significance, following 10ppm SO_2 exposure was detected. In rats on a standard diet, increasing SO_2 concentrations produced statistically significant 46% and 73% increases of plasma triglycerides, respectively, in 5 and 10 ppm SO_2 exposed rats.

Plasma immunoreactive insulin levels, determined in rats on a standard diet, showed a statistically significant dose-dependent reduction of 46 and 64%, respectively, after 5 and 10 ppm SO_2 exposure.

Liver lipid analyses revealed a marked dose-related increase of the TG (triglyceride) content in rats on a standard diet, reaching statistical significance after 10 ppm SO_2 exposure.

Effect of SO_2 exposure in rats fed HC diet
The body weight gain, recorded at the end of the experimental period, was reduced in HC rats exposed to SO_2, compared to respective controls. In these animals an increasing feed consumption, reaching statistical significance, following 10 ppm SO_2 exposure was also detected.

Plasma immunoreactive insulin levels, determined in rats on the HC diet, showed a statistically significant dose-dependent reductions (-21 and -33%, respectively, after 5 and 10ppm SO_2 exposure).

Statistically significant increases in liver weight were detected in 10ppm SO_2 exposed HC rats. Liver lipid analyses revealed a marked dose-related increase in the TG content in these animals, reaching statistical significance after 10 ppm SO_2 exposure.

Effect of the exposure to SO_2 of standard and HC diets
In order to assess whether the exposure of the diets to SO_2 could alter them, thus being responsible for the changes in the examined plasma parameters, an experiment was performed where control rats, not belonging to the main experiment, were given standard and HC diets pre-exposed for 3 days to 10ppm SO_2.No variations in the evaluated parameters of non exposed diets were detected, thus suggesting that the exposure of the diets to SO_2 was not responsible for the described plasma and tissue effects of exposed rats.

Effect of SO_2 exposure in STZ-diabetic rats
A positive trend in feed consumption versus diabetic controls was observed, which reached statistical significance in 10ppm SO_2 exposed rats.
Diabetic rats showed a marked liver weight reduction, respectively -21 and 27% following 5 and 10 ppm SO_2; a marked dose-dependent decrease in liver triglyceride content was detected in exposed STZ-diabetic animals. Statistically significant, but not dose dependent, reductions of the liver esterified cholesterol content were finally detected in 5 and 10ppm SO_2 exposed diabetics.

*Statistically significant

Author's Conclusions:

In conclusion, the reported data point out to a systemic effect of SO_2 in animal models of frequent human pathologies, such as hypercholesterolemia and diabetes. Available data do not permit, as of now, transposition of data from experiments carried out in animals to man, since people living urban areas are exposed to different air pollutants, the effect of which on health may be combined or superimposed.

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (+) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(+)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined?

	(+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+)Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+)Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-)Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (+)Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (+)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(+/-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (+)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (-) Were test animals randomly assigned to exposure groups? (+/-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment)

	(+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (+) Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

27 rats and appropriate controls were exposed to 5 and 10 ppm SO_2 in order to determine what biochemical effect this exposure would have on the cholesterol level of normal and diabetic rats. Results indicate that a systemic effect of SO_2 exposure on lipid metabolism in rats fed diets with different lipid content as well as in STZ-diabetics exists. These effects are observed at relatively low SO_2 concentrations that do no induce perceptible alterations of lung function. The SO_2 exposure in the study appears to affect mainly TG metabolism, with a statistically significant, dose-dependent rise of plasma and liver TG contents both in rats on standard and HC diets. The study could show no correlation between SO_2 inhalation and changes in lipid metabolism, however, making it difficult to explain the data presented. Also, since both the animals and the diets are exposed to SO_2, it is difficult to answer whether gas exposure itself or alterations in lipid/protein components of the diets, could be responsible for the observed changes.

The reported data point to a systemic effect of SO_2 in animal models of frequent human pathologies, such as hypercholesterolemia and diabetes. In both standard and HC rats, SO_2 exposure at sub-chronic doses led to a significant increase in plasma and liver triglyceride content. In contrast, diabetic rats had lower plasma and liver triglyceride content when exposed to SO_2.

Confidence Index:

The study did try to limit confounding variables by using same sex and weight rats. Experimental conditions for both exposure and assessment were well defined and should be easily reproducible. A gradient of exposures was used to determine the effects of SO_2 in rats on various diets. The diets were used to assume two human pathologies: diabetes and hypercholesterolemia although only two exposure levels were examined. It was also unclear as to whether subjects were randomly allocated to groups. The data clearly show that diabetic rats respond differently to SO_2 exposure (decreased plasma and tissue lipid contents) than rats on standard or high cholesterol diets (increased plasma and tissue triglycerided content) and raise the necessity to evaluate different organs, which normally are not believed to be target sites for SO_2.

Review – Confidence index ranking:

High

Author: Korpas J. Widdicombe JG.	**Internal ID: 153**
Title: Defensive Respiratory Reflexes in Ferrets.	
Year: 1983	
Abstract: We have studied defensive reflexes in the ferret. As for the mouse, this species lacks a cough reflex on mechanical stimulation of the tracheal mucosa, and this may be related to the lack of nerves and mucus-secreting cells in the tracheal epithelia of these species. The bronchial cough reflex is similar to that of guinea pigs and rabbits, and the laryngeal reflexes are similar to those in rats.	
Objective: n/a	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
1ml of 20% (v/v) SO2	**Single exposure**	**ferret**	**n/a**	**n/a 0.44-1.53kg**	**Male**	**15**	**n/a**	**n/a**	**n/a**

Observations:

"Coughing in response to SO_2 was weaker than that to mechanical stimulation, and was easier to elicit from the larynx than from the bronchi."

*Statistically significant

Author's Conclusions:

"Chemical stimulation, in spite of the fact that it might activate receptors throughout all the airways, produced a weaker cough than did mechanical stimulation. There was none of the hyperventilation which is characteristic of the response to SO_2 in the rat. As with mechanical stimuli, for SO_2 the larynx was particularly sensitive."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health

	☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* ±✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? -✎ Was the duration of exposure precisely defined? -✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	-✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* ±✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +/-✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration?

	☐ *Guideline: 5 days* -✏ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? -✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✏ Was a control group assayed? ±✏ Is the control regime appropriate? -✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? ±✏ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✏ Were accepted assessment methods employed? ±✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? -✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✏ Was individual data reported for each test animal?
G. Data analysis:	+✏ Are the statistical methods used appropriate? ±✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective? +✏ Were assumptions reasonable? +✏ Are the methods reproducible? +✏ Did either the study design or the statistical analysis limit confounding variables? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The purpose of this study was not to test the effects of differing gradients of SO_2 on the pulmonary function of ferrets. This study was designed to demonstrate a linkage between airway mucus secretions and the cough reflex, use of SO_2 was incidental as it was an easily obtainable irritant, but not deadly gas in low concentrations. If similar results to this animal model are seen in humans, respiratory irritation to chemical exposures such as SO_2 may be better understood.

Confidence index:

This study poorly reported the study animals' characteristics, exposure conditions, and the study of SO_2 exposure was only a small part of the research done. Only one endpoint was measured – expiration and inspiration intensity of cough. Lack of information on ferret source, reagent source, exposure duration, lab diet, acclimation period significantly lower the confidence in this study. As such, the results of this study must be viewed with caution.

Review – Confidence index ranking:

Moderate to low

Author: Kahana LM. Aronovitch M.	**Internal ID:** 155
Title: Pulmonary surface tension after sulfur dioxide exposure.	
Year: 1968	
Abstract: n/a	
Objective: To determine if pulmonary surface tension is affected by sulfur dioxide exposure	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
800 **1226**	**3 hrs** **2 hrs**	**Rat**	**Albino**	**Young**	**Male**	**26**	**n/a**	**n/a**	**n/a**

Observations:

At 800ppm for 3 hours: "…no signs of respiratory distress, and the lungs, when examined grossly, showed no pathologic changes. Nevertheless, there was a small but significant reduction in the mean values for both maximal and minimal surface tension."

At 1225ppm for 3 hours: "Changes suggesting pulmonary edema were observed in the SO_2-1200 group, including a significant increase in the mean weight of the lungs. Severe and persistent dyspnea developed in 2 of the rats, and one had foamy secretions in the trachea. The other animals had lesser degrees of respiratory difficulty of a transient nature… In the surface balance, in contrast to the SO_2-800 group, the lung extracts showed a more uniform and much more striking drop in surface tensions. Mean maximal tension was reduced from the control value to 37.2 dynes per cm ($p<0.001$) and minimal tension to 11.7 dynes per cm ($p<0.005$)." (from 44.8 and 18.4 dynes per cm, respectively).

*Statistically significant

Author's Conclusions:

"In Summary: The surface tension of lung extracts has been measured with the modified Wilhelmy balance after short, high level exposure of rats to SO_2. The findings were interpreted as a demonstrating an increase of surfactant activity that might suggest that this increase in surfactant activity have been caused either by increased production of surface-active substance or by some qualitative change in it. Results of an earlier study is ass associated with a decrease in lung elastic recoil, and the possibility was considered that such changes might be related to the pathogenesis of emphysema."

Review – Assessment

A. Test animals:	-✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study?

	+a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex -d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* -✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +-✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? -✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	+/-✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* +✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water*

	may be withheld optionally.
D. Equipment:	-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* -✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? -✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? -✎ Is the control regime appropriate? -✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? -✎ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? ±✎ Were assessment measures sufficiently sensitive? +/-✎ Was the nature and severity of symptoms graded against an explicitly defined scale? ±✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? -✎ Are the results novel? -✎ Was the design of the study appropriate to investigate the primary objective? -✎ Were assumptions reasonable?

	-✎ Are the methods reproducible? -✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study attempted to determine the changes in pulmonary surface tension after acute sulfur dioxide exposure. If changes in pulmonary surface tension occur, it may be possible to relate pulmonary emphysema to changes produced by inhaled irritants. In the SO_2-800 group, there were no signs of respiratory distress and the lungs showed no pathologic changes. There was a small but significant reduction in the mean values for both maximal and minimal surface tension. Changes suggesting pulmonary edema were observed in the SO_2 1200 group were observed. Compared to the $SO_2$800 group, the lung extracts showed a more uniform and much more striking drop in surface tensions. The changes in surface tension may provide supporting information as to the biological mechanism of SO_2 exposure.

Confidence index:

Only 6 rats were used for the SO_2-1200 level and were only exposed for 2 hours. The SO_2-800 group and the controls were in the chambers for 3 hours. Only male rats were used. The exposure levels were extremely high and there was no justification for the doses used. These doses may have been too high, resulting in the normal mechanisms of the body to be overwhelmed and resulting in inappropriate conclusions. There was considerable information missing regarding the animals and study design (see above). In the $SO_2$1200 group, severe and persistent dyspnea developed in 2 of the rats and one had foamy secretions in the trachea. The other animals had lesser degrees of respiratory difficulty of a transient nature. These differences could indicate interspecies variability. The authors did not report how the rats were tested for respiratory distress. The determination of the surface tension was a very involved process and the accuracy and precision of the results will depend on the personnel performing the experiment. No information is given regarding the qualifications of the technicians. As the authors point out, the analysis of lung extracts with the surface balance is primarily a qualitative procedure and the direction of change is perhaps of more importance than the degree of change.

Review – Confidence index ranking:

Moderate to low

Author: Haider SS. Hasan M. Hasan SN. Khan SR. Ali SF.	**Internal ID: 159**

Title: Regional effects of sulfur dioxide exposure on the guinea pig brain lipids, lipid peroxidation and lipase activity.

Year: 1981

Abstract: Experiments to evaluate the neurochemical action of gaseous pollutants were performed in a specifically designed inhalation chamber. Exposure of guinea pigs to 10 ppm of SO_2 for one hour daily for 21 days produced a significant depletion of total lipids and free fatty acids in all brain regions. Phospholipid and cholesterol content of the cerebellum and brain stem were also decreased, but were increased in cerebral hemisphere. Esterified fatty acids were depleted in the cerebral hemisphere and brain stem, but exhibited increment in the cerebellum. The rate of lipid peroxidation and the activity of lipase were increased significantly in all the regions of the brain. The results clearly indicate that SO_2-exposure induces degradation of brain lipids. Interestingly, the lipid contents are affected differentially in the various parts of the brain.

Objective: To examine effects of SO_2 exposure on the guinea pig brain, in terms of lipids and lipase activity.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
10ppm	**1hr/d for 21d**	**Guinea pig**	**n/a**	**Adult (200-500 grams)**	**Male**	**24**	**n/a**	**n/a**	**10ppm**

Observations:

"The exposure of guinea pigs to SO_2 led to signs of nasopharyngitis, somnolence, staggering, itching, preening, skin and eye irritation… A statistically significant decrement in total lipid content of the cerebral hemisphere and brain stem were found ($p<0.001$ and $p>0.05$)… The levels of phospholipids were significantly decreased in the cerebral hemisphere ($p<0.001$) and decreased in the cerebellum ($p<0.05$). The concentration of cholesterol showed significant increase in cerebral hemisphere ($p<0.05$); but the cholesterol concentration was decreased significantly in the cerebellum and brain stem ($p<0.05$). The values of free fatty acids were significantly diminished in cerebral hemisphere, cerebellum, and brain stem ($p<0.05$. $p<0.02$, $p<0.001$). Esterified fatty acids were depleted significantly in cerebral hemisphere and brain stem ($p<0.05$ and $p<0.001$). Interestingly, these levels were increased in the cerebellum ($p<0.001$). The rate of lipid peroxidation and the activity of lipase were significantly increased in all regions of the brain ($p<0.05$, $p<0.001$)."

*Statistically significant

Author's Conclusions:

"Exposure of guinea pigs to SO_2 (10ppm) has shown alteration in the regional lipid profiles of the guinea pig brain. The concentration of total lipis was depleted significantly in all brain regions except the cerebellum. In the cerebral hemisphere a significant decrease in the phospholipids and cholesterol levels was matched by depletion of free and esterified fatty acids."

Review – Assessment:

A. Test animals:	+✏ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✏ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✏ Were the chosen exposure levels appropriate to investigate the primary objective? -✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? ±✏ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? -✏ Was evidence provided to justify the dosing regime? +✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✏ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✏ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✏ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.*

	+✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✏ Was a full description of the monitoring devices provided? +/-✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✏ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? ±✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? +✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✏ Was individual data reported for each test animal?
G. Data analysis:	+✏ Are the statistical methods used appropriate? +✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective?

	+✓ Were assumptions reasonable? +✓ Are the methods reproducible? -✓ Did either the study design or the statistical analysis limit confounding variables? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Exposure of guinea pigs to 10 ppm of SO_2 for one hour daily for 21 days produced a significant depletion of total lipids and free fatty acids in all brain regions. Phospholipid and cholesterol content of the cerebellum and brain stem were also decreased, but were increased in cerebral hemisphere. Esterified fatty acids were depleted in the cerebral hemisphere and brain stem, but exhibited increment in the cerebellum. The rate of lipid peroxidation and the activity of lipase were increased significantly in all the regions of the brain. The results clearly indicate that SO_2-exposure induces degradation of brain lipids. Interestingly, the lipid contents are affected differentially in the various parts of the brain. The study of SO_2 effects on brain chemistry is relatively new, and may have profound implications, particularly if the results seen in this animal model are also demonstrated in humans.

Confidence index:

Novel study providing information on effect of SO_2 on brain lipids. Animal exposure conditions were well defined and well established methods were used to investigate brain lipid composition, lipid peroxidation and lipase activity. Study should be easily reproducible. Same sex animals used for study, if there was randomization of controls and treated this will limit weight as a confounding variable. Sample sizes are adequate as groups of 12 were used for each of the treatment and control groups. While some animal characteristics and detail from the methods section may be lacking, it seems unlikely that they would affect the quality of the study.

Review – Confidence index ranking:

High to moderate

Author: Hanacek J. Adamicova K. Briestenska J. Jankovska D. **Internal ID: 161**

Title: Cough reflex in rabbits 24-h and 48-h after sulphur dioxide breathing.

Year: 1991

Abstract: The cough reflex elicitability (CRE), cough reflex strength (CRS) and Hering-Breuer inflation reflex (HBIR) were studied in 51 anaesthetized (Pentobarbital Spofa, 30 mg/kg, i.v.) female rabbits 24-h and 48-h after SO_2 breathing. To provoke cough, the interior of the trachea and carina were stroked with a polyethylene catheter. To elicit the HBIR, the lungs were inflated to 1.0 kPa intratracheal pressure. Intrapleural and systemic blood pressures were recorded. The CRE, CRS and HBIR obtained 24-h and 48-h after SO_2 breathing were compared with correspondent values of control animals. It was found, that CRE and HBIR were fully recovered 24-h after SO_2 breathing, but the CRS was still decreased, however, there were no significant differences in CRE, CRS and HBIR between animals 48-h after SO_2 breathing and control animals. It can be concluded, that decrease of the CRS 24-h after SO_2 breathing is not due to slowly adapting stretch receptors block of airways.

Objective: "The present study is devoted to solve the question whether cough reflex inhibition which is present in rabbits immediately and up to 2 hours after SO_2 breathing, will be still present 24h and 48h after SO2 breathing was stopped."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
200-300ppm	**10-20min**	**rabbit**	**n/a**	**Adult (2.4-4.2 kg)**	**female**	**51**	**n/a**	**n/a**	**n/a**

Observations:

"We have found that 24h after SO_2 breathing all of the tested animals were able to cough after each airway stimulation. That means that elicitability of the cough reflex was fully recovered and it was almost the same as in control animals. HBIR was recovered too. At this time, the CRS was still decreased, however. Number of cough efforts (NE) was decreased to 65%, intensity of maximum cough effort (IME) have reached 49-82% of control values and intensity of cough attack (IA) was decreased to 53-71% of control values. The expiratory values of IME and IA were decreased more than inspiratory ones… It is apparent that expiratory values of IME and IA in rabbits 24-h after SO_2 breathing are significantly decreased ($p<0.01$) compared to the control group. Differences in NE and NE/min are not significant."

"48h after SO_2 breathing, the elicitability of the cough reflex and HBIR strength were still normal, but in quantitative values of the cough reflex we can see certain decrease. These differences, however, are not significant."

*Statistically significant

Author's Conclusions:

"It is important to emphasize that 24h after SO_2 breathing the HBIR as well as the elicitability of the cough reflex was fully recovered, too, but CRS was still decreased. What could be the reason for getting these results? The function of slowly adapting stretch receptors of the airways is not changed at this time, as we can judge it from HBIR strength. It has been described that the function of rapidly adapting stretch receptors are not changed significantly after SO_2 breathing, either. It seems to be clear that neither of these two groups of airway receptors can be responsible CRS decreasing 24h after SO_2 breathing. Could the C-fibre receptors of the airways be responsible? Our opinion is – yes."

Review – Assessment:

A. Test animals:	+✏ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✏ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✏ Were the chosen exposure levels appropriate to investigate the primary objective? +✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? -✏ Was the duration of exposure precisely defined? -✏ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* ±✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? -✏ Was evidence provided to justify the dosing regime? -✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?

C. Housing/Feeding:	±✏ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✏ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✏ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	-✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* -✏ Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✏ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? +/-✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? -✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✏ Was individual data reported for each test animal?

G. Data analysis:	+✏ Are the statistical methods used appropriate? +✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective? +✏ Were assumptions reasonable? +✏ Are the methods reproducible? +✏ Did either the study design or the statistical analysis limit confounding variables? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

It was found, that cough reflex elicitability and Hering-Breuer inflation reflex were fully recovered 24-h after SO_2 breathing, but the cough reflex strength was still decreased, however, there were no significant differences in cough reflex elicitability, cough reflex strength and Hering-Breuer inflation reflex between animals 48-h after SO_2 breathing and control animals. The implications of this research are a better understanding of respiratory response to SO_2 in humans.

Confidence index:

The methods of exposure (concentration of SO_2 and duration of exposure) appear to be questionable, as neither parameter seems to be accurately defined. Sample size is large (n=51), but the lack of consistent exposure circumstance may adversely affect the data that were collected. Only one endpoint was studied – cough reflex following SO_2 exposure. Paucity of references considering this is published in 1991.

Review – Confidence index ranking:

Low to Moderate

Author: Islam, M.S., Vastag, E., Ulmer, W.T.	**Internal ID:** 162

Title: Sulphur-Dioxide Induced Bronchial Hyperreactivity against Acetylcholine

Year: 1972

Abstract: The effect of sulphur-dioxide (SO_2) on the bronchial reactivity against acetylcholine (ACH) is carried out on 25 dogs. The dogs were exposed initially to acetylcholine aerosol, then to SO_2 for 1 hour and again to ACH. 3 such cycles were repeated., The concentration of SO_2 used were 1, 2, 5 and 10ppm and the controls were given room air in place of SO_2. The parameters studied were intrathoracic pressure as the indicator of bronchoconstriction, blood oxygen and carbon-dioxide, tidal volume, respiratory rates and arterial blood pressure. All the SO_2 exposed animals have shown a significant increase of bronchial sensitivity than those of the controls. Maximum sensitization was induced by 2ppm SO_2. The reaction provoked by 1 and 5 ppm were almost of the same order and little smaller than 2 ppm but greater than those produced by 10ppm. The peak reactions against ACH observed for 1, 2 and 5ppm were 3, 2 and 1 hr of exposure, respectively. After attaining the peak points, the reactions declined in all the 3 groups. But no such tend was obtained for those animals exposed to 10 ppm of SO_2.

Objective: To examine the role of SO_2 in sensitizing the bronchial system against provocative irritants which could be the cause of delayed reactions.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0, 1, 2, 5, 10 ppm SO_2	60 minutes (3x)	Dogs	Boxer	Unknown	Male and female	25 (2 control groups (n=4 and n=3) and 4 experimental groups (n=18 total))	unknown	unknown	unknown

Observations:

All the SO_2 exposed animals showed a significant increase of bronchial sensitivity than those of the controls. Maximum sensitization was induced by 2 ppm. The reaction provoked by 1 and 5 ppm were almost of the same order and little smaller than 2 ppm but greater than those produced by 10 ppm. The peak reactions against ACH observed for 1,2 and 5 ppm were 3, 2 and 1 hr of exposure, respectively. After attaining the peak points, the reactions declined in all the 3 groups. But no such trend was obtained for those animals exposed to 10 ppm of SO_2.

*Statistically significant

Author's Conclusions:

Bronchoconstriction caused by SO_2 was both dose and time dependent. The effect of 10 ppm SO_2 on bronchial sensitivity is somewhat conflicting as it is considerably smaller than that produced by lower concentrations of SO_2 and does not reveal the peak reaction point. Prior inhalation of small concentrations of SO_2 is able to potentiate the ACH induced changes in bronchial reactivity.

Review – Assessment:

A. Test animals:	(-)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+/-)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-)) Weight variation *Guideline: Below 20% variation* (-) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary

	objective? (-)Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-)Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-)Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water May be withheld optionally.*
D. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (+/-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+)Was a control group assayed? (+) Is the control regime appropriate? (-)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*

F. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(-) Are the statistical methods used appropriate? (-)Are confidence intervals reported? (+/-) Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

A gradation of SO_2 concentrations was used to stimulate bronchial hyperreactivity in dogs. This study does have some relevance to human exposure guidelines, as it supports a weight of evidence argument for specific levels of SO_2 exposure. However, lack of information in certain areas decreases the study's credibility. In the study, the maximum sensitization took place at 2ppm SO_2. There was no statistical analysis, so it is difficult to determine whether the differences in response to varying concentrations of SO_2 are significant.

Confidence Index:
While information was available on breed of animal, number and manner of exposure, lack of information about source of animals, animal health, source of reagents and in-depth statistical analysis detract from the confidence in this study. Furthermore, there was no justification for the use of dogs. The objective of the study is to determine the role of SO_2 in sensitizing the bronchial system against provocative irritants. The dogs were exposed to the ACH before SO_2. Perhaps, it would have made sense to test another group of animals with the exposures reversed for comparison purposes. The ACH exposure was only 5 minutes and then SO_2 exposure took place immediately after. The length of the follow-up period was inadequate. The exposure duration of each ACH administration was only 5 minutes. The animals were not randomly assigned to exposure groups. Only male dogs were used and the weight variation was greater than 20%. The sample sizes per exposure and control group were too small. Also, each exposure group differed in size. There was no statistical analysis. The data is just analyzed in percentage changes. The differences between the SO_2 responses and the control responses are stated as being " definitely caused by SO_2". But, no justification or further analysis is done. Finally, there was no preliminary testing to determine appropriate levels of SO_2 or ACH.

Review – Confidence index ranking:

Low to moderate

Author: Knorst, M.M., Kienast, K., Riechelmann, H., Muller-Quernheim, J., Ferlinz, R.	**Internal ID:** 164

Title: Effect of sulfur dioxide on mucociliary activity and ciliary beat frequency in guinea pig trachea

Year: 1994

Abstract: The effects of 30 min exposure to sulfur dioxide on mucociliary activity (MCA) and ciliary beat frequency (CBF) were studied in 31 guinea pig tracheas. MCA was measured by recording the light reflected from ciliated mucous membranes using an infrared bar code reader. CBF of single ciliated cells obtained by brushing was measured with phase-contrast microscopy. Each tracheal sample was exposed to SO_2 at concentrations ranging from 2.5 to 12.5 ppm, or to air for control purposes. MCA and CBF were measured before and immediately after gas exposure. A reduction in mean MCA of 63% ($P = 0.0007$) and statistically insignificant changes in CBF ($P > 0.05$) were recorded at concentrations of 2.5 ppm SO_2. Higher SO_2 concentrations caused a further impairment of MCA as well as a dose-dependent decrease in CBF ($P = 0.002$). A concentration of 12.5 ppm SO_2 induced a decrease from baseline values of approximately 80% in mean MCA and of roughly 70% in mean CBF. This study demonstrates a dose-dependent SO_2-induced decrease in MCA of guinea pig tracheas. The decrease in MCA was associated with an impairment of CBF only at SO_2 concentrations higher than 5.0 ppm.

Objective: To investigate the short-term effect of increased SO_2 concentrations ranging from 2.5 to 12.5 ppm on CBF and mucociliary activity (MCA) of guinea pig trachea. The measurement of CBF of single ciliated cells provided information on the performance of the cilia, while analysis of MCA was performed to determine the interaction between cilia and mucus.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
2.5, 5, 7.5, 10, 12.5	30 minutes each	Guinea Pig	No info	No info	No info	31 (Divided into 6 groups) Control=7 Experimental Groups: N=6 N=6 N=4 N=4 N=4	healthy	1ppm	N/A

Observations:

There was no significant difference in mean MCA among the six groups. The control exposure of the trachea to air yielded no significant changes in MCA.

Exposure to SO_2 up to 12.5ppm induced a significant dose-dependent decrease in MCA (p=0.009). There were no significant differences among the six groups in the mean CBF. After SO_2 exposures there was a significant decrease in the CBF of the tracheal samples (p=0.002) with a strong negative correlation between CBF and SO_2 concentration.
*Statistically significant

Author's Conclusions:

In summary, the present study demonstrates a significant mucociliary dysfunction of guinea pig trachea after short-term exposure to SO_2. Further studies will be necessary to elucidate the mechanisms responsible for the alterations in mucociliary clearance induced by SO_2.

Review – Assessment:

A. Test animals:	(-)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (-)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary

	objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+/-)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+/-)Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark, 12 to 15 changes/h; 19%* (N/A) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (N/A) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (No) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (N/A) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(N/A) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+/-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(N/A) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (+/-) Was the method of randomization defined? (*i.e.*, information pertaining to

	the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (N/A) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+/-)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (No) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+/-) Are the results novel? (+/-) Was the design of the study appropriate to investigate the primary objective? (+/-) Were assumptions reasonable? (+/-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+/-) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study was conducted in order to analyze the effects of short-term exposure to SO_2 on CBF and MCA of guinea pig trachea. The findings show a decrease in MCA of guinea pig trachea after SO_2 exposure. The tracheas were directly exposed to SO_2 resulting in a greater amount of gas reaching the surfaces than on in vivo inhalation of the same concentration of SO_2.

Confidence index:

This study investigates the mechanism involved in SO_2 induced alteration of mucociliary clearance by measuring ciliary beat frequency and mucociliary activity. These parameters were measured using appropriate equipment (not subjective). Findings are in agreement with other published studies in other animals. Additional parameters should have been studied to identify a mechanism for the mucociliary dysfunction – this may be a limitation of using isolated guinea pig trachea. Experimental design was simple and well defined – should be easily reproducible. However, there was no indication that the animals were randomly assigned and the sample size was inadequate to produce statistically significant results. Furthermore, no information was given on the age or sex of the guinea pigs.

Review – Confidence index ranking:

Low

DRAFT

Author: Gross, P., Rinehart, W.E., Smyth, H.F., Burton, K.J.	**Internal ID:** 166
Title: Morphologic Criteria of Pulmonary Edema	
Year: 1969	
Abstract: The usual morphologic criterion of alveolar edema is the presence of homogeneous acidophilic material in the air spaces. When interstitial pulmonary edema is present, it is often assumed that alveolar edema is associated even though there may not be any demonstrable proteinaceous material in the air spaces. Findings in rat lungs are described that serve as additional criteria of alveolar edema. These are separations of surface epithelium from the alveolar septum to form blebs and larger clear spaces on the alveolar wall, finally resulting in cast-off membranes completely free in the air spaces.	
Objective: Recently, in studying lung sections of rats killed or dying two days or less after high exposures to sulfur dioxide SO_2, findings suggesting the presence of alveolar edema were made that had apparently escaped notice in previous studies. It is the purpose of this paper to describe these findings that are considered to be additional morphologic evidence of alveolar edema.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
2500-4400 ppm	15-minute exposures	Rats	Wistar-derived albino	No info	Male	7	No info	N/A	N/A

Observations:

There were separations of the surface epithelium from the alveolar septum, thereby forming clear spaces of various sizes and shapes delimited on the luminal side by an extremely thin membrane and on the opposite side by the alveolar septum.
*Statistically significant

Author's Conclusions:

The detachment of alveolar surface epithelium by edema suggests that the development of edema was extremely rapid, which did not allow the edema fluid to escape as through a sieve, but tore the sieve from the attachment.

Review – Assessment:

A. Test animals:	(-)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study?

	(+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (-) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+/-)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (-) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated?

	Guideline: Groupings should permit clear observation of each animal. (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (+/-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? (-) Were test animals randomly assigned to exposure groups? (N/A) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (-) Was a control group assayed? (N/A) Is the control regime appropriate? (-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined? (+/-)Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own

	conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(N/A) Are the statistical methods used appropriate? (N/A) Are confidence intervals reported? (N/A) Did the investigator appropriately interpret the statistics? (N/A) Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (+/-) Were assumptions reasonable? (+/-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+/-) Do the conclusions make biological sense? (N/A) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The observations made in this study were that exposure to very high levels of SO_2 in rats caused the separation of surface epithelium from the alveolar septum to for blebs and larger clear spaces on the alveolar wall, resulting in cast-off membranes completely free in the air spaces. The concentrations of SO_2 used are not clinically significant as far as humans are concerned. The exposure levels ranged from 2500-4400 ppm.

Confidence index:

Sample size is small, exposure concentrations and conditions are not well defined. Lack of information as to animal source, health, diet, lack of source information of reagents, exposure device, number of exposure levels and lack of statistical analysis all detract from this study. Furthermore, some of the exposure levels were too high, causing some of the rats to die within 2 hours. Finally, no control group was assayed.

Review – Confidence index ranking:

Low

Author: Cho YW. Samanek M. Aviado DM.	**Internal ID: 167**
Title: Differences in the effects of inhalation of sulfur dioxide and cigarette smoke.	
Year: 1968	
Abstract: In the anesthetized dog the inhalation of sulfur dioxide caused bronchoconstriction, which persisted even after acute and chronic denervation. The basic mechanisms responsible for this bronchoconstriction as well as the accompanying bronchial arterial vasodilation are different from those activated during the inhalation of cigarette smoke.	
Objective: "The experiments reported in this article were performed to determine if sulfur dioxide could influence the airways by way of the three listed mechanisms. The procedure was to isolate one lobe of the lung demonstrating each of the following important aspects: ventilation, pulmonary blood flow, and bronchial blood flow."	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0.01-1.0% SO2	**0.1-6.0 min**	**dog**	**mongrel**	**n/a 17-30kg**	**n/a**	**8**	**n/a**	**n/a**	**0.01%**

Observations:

"The inhalation of sulfur dioxide ranging in concentration from 0.01% to 1% initiated bronchoconstriction in all the anesthetized dogs tested."

"In almost all instances, the bronchoconstriction was limited to the exposed lobes and did not extend to the other lobes which remained free of sulfur dioxide."

"The exposure of the perfused lobe to sulfur dioxide did not cause an elevation of the perfusion pressure."

"In each of the three preparations including the perfusion of the right bronchial artery, the inhalation of sulfur dioxide in most of the lobes caused a fall in bronchial arterial perfusion pressure."

*Statistically significant

Author's Conclusions:

"The four reasons that have just been enumerated support the conclusion that sulfur dioxide does not influence the lung in the same manner as cigarette smoke. The lack of participation of interlobar and intralobar reflex mechanisms during the exposure of the lob to sulfur dioxide indicate that other mechanisms are more important. The participation of interlobar reflex can be completely excluded but not an intralobar nervous mechanism. It is conceivable that sulfur dioxide was simultaneously stimulating the intralobar nervous and nonnervous mechanisms. After administration of atropine or performance of a procedure causing chronic denervation, the nervous mechanisms were eliminated but the bronchoconstrictor response persisted by means of the nonnervous mechanism. Ultimate identification of the latter and its exclusion are essential to finally exclude the participation of a nervous intralobar mechanism. Unless this is completed, the exclusion of a nervous mechanism is questionable."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex -d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ±✎ Were both nominal and actual exposure concentrations recorded? ±✎ Was the duration of exposure appropriate to investigate the primary objective? -✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate?

	☐ *Guideline: Animals may be grouped by sex, or individually.* ±✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	-✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* -✏ Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✏ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) -✏ Was a control group assayed? ±✏ Is the control regime appropriate? -✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? -✏ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? -✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? -✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✏ Was individual data reported for each test animal?
G. Data analysis:	-✏ Are the statistical methods used appropriate? -✏ Are confidence intervals reported? ±✏ Did the investigator appropriately interpret the statistics? ±✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel?

	±✏ Was the design of the study appropriate to investigate the primary objective? +✏ Were assumptions reasonable? +-✏ Are the methods reproducible? -✏ Did either the study design or the statistical analysis limit confounding variables? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

In the anesthetized dog the inhalation of sulfur dioxide caused bronchoconstriction which persisted even after acute and chronic denervation. The basic mechanisms for the relaxation of the vascular smooth muscle and the contraction of the bronchial smooth muscle during exposure to SO_2 have not been identified.

Confidence index:

The methods are described rather poorly, particularly the animal characteristics and methods of SO_2-gas generation and exposure. Lacked information as to source of dog, strain of dog was inappropriate, number of animals did not generate meaningful statistical data, lack of reagent source, lack of information as to exposure apparatus. No statistical analysis, but then again no controls for surgical procedures. However, this paper was written in the 1960s and the level of descriptiveness seems similar to other papers from this period.

Review – Confidence index ranking:

Low

Author: Frank NR. Yoder RE. Brain JD. Yokoyama E. **Internal ID: 169**

Title: SO_2 (^{35}S labeled) absorption by the nose and mouth under conditions of varying concentration and flow.

Year: 1969

Abstract: Sulfur dioxide ^{35}S ($^{35}SO_2$) was administered to the surgically isolated upper airways of anesthetized dogs via a mask with separated connections to the nose and mouth. The $^{35}SO_2$ that passed through the upper airways was monitored continuously; nearly complete removal could be measured to within several hundredths of a percent. Nasal uptake of the incoming gas (1 to 50ppm) exceeded 99% at a flow of 3.5L/min and fell only several percent when flow was increased tenfold (1, 10ppm). Uptake by the mouth averaged more than 95% at a flow of of 3.5L/min (1, 10ppm), but fell to under 50% when flow was increased tenfold. Following penetration of mouth or nose by $^{35}SO_2$, desorption of the gas from the mucosa could be demonstrated more than 25min after the end of exposure.

Objective: "The object of the present investigation was to furnish this information (whether the concentration of gas or the ventilatory flow rate might be more important in determining the degree of SO_2 by either oral or nasal pathways) by making a direct comparison of the absorptive capacities of the mouth and nose for $^{35}SO2$ under the conditions in which the gaseous concentration and the rate of administration could be changed independently."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
1-50ppm	**1.5-5.0 min**	**Dog**	**n/a**	**Adult (15-18kg)**	**n/a**	7	**n/a**	**n/a**	**n/a**

Observations:

"The nose adsorbed virtually all of the $^{35}SO_2$ (1 to 50ppm) administered at 3.5L/min for 5min. Whatever nasal penetration did occur amounted, at most, to about 2 to 3 x 10^{-3} ppm."

"Penetration of the mouth, unlike the nose, was greater at the higher concentration. The average was 0.44% at 1ppm (four animals, eight exposures), and 3.7% at 10ppm (four animals, six exposures)."

"At a flow of 35L/min, penetration of the mouth by $^{35}SO_2$ was both rapid and marked; at 1ppm the average penetration was 66% after only 1 to 3 minutes (three dogs, four exposures)."

"Following exposures in which $^{35}SO_2$ penetrated either the nose or mouth, the administration of $^{35}SO_2$-free compressed air to the same segment was associated with desorption of radioactive gas from the mucosal surfaces… desorption was virtually confined to the mouth; when air was diverted through the unexposed nose, desorption became negligible. The release of the gas from the mouth was still demonstrable more than 25min after the end of exposure."

*Statistically significant

Author's Conclusions:

"…its suggests that the amount of SO_2 reaching the larynx and lower airways will depend more on the mode of breathing – for example, whether it be nasal or oral, fast or slow – than it will on fluctuations in the level of SO_2 in the environment. Thus, the high flow rates and obligatory mouth breathing typical of exercise and heavy labor are likely to result in an increased exposure of the lower airways to SO_2 that is out of proportion to the associated increase in minute ventilation. A third significant finding was of the continuous release of $^{35}SO_2$ from the mucosa into the airstream following the cessation of exposure. In some experiments, desorption of $^{35}SO_2$ was still evident after 25 to 30minutes of flushing with ambient air. A similar occurrence has been reported for human subjects."

Review – Assessment:

A. Test animals:	+✏ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✏ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* +b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✏ Were the chosen exposure levels appropriate to investigate the primary objective? -✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? -✏ Was the duration of exposure precisely defined? ±✏ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? -✏ Was evidence provided to justify the dosing regime? -✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✏ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate?

	☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* ±✏ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✏ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* ±✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✏ Was a full description of the monitoring devices provided? +✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✏ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? -✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) -✏ Was a control group assayed? +/-✏ Is the control regime appropriate? ±✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? -✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? +✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✏ Was individual data reported for each test animal?
G. Data analysis:	-✏ Are the statistical methods used appropriate? -✏ Are confidence intervals reported?

	±✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? -✎ Are the methods reproducible? -✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Eight dogs were exposed to a range of concentrations of $^{35}SO_2$ ranging from 1-50 ppm at various amounts of flow (3.5, 35l/min). the study demonstrated that most SO_2 uptake was in the nose rather than the mouth, but penetration of the SO2 into the tissues was greater in the mouth. This suggests that the amount of SO_2 reaching the larynx and lower airways will depend more on the mode of breathing than on fluctuations in the level of SO_2 in the environment. ie shallow, nasal breathing will decrease exposure of lower airways to SO_2.

Confidence index:

The methods of SO_2 gas generation are rather vague; lack of information as to animal source, strain/breed, reagent source, statistical analysis, defined exposure levels and frequency all limit confidence in this study.. The authors mention problems with the gas-generation system, that the walls of the container adsorbed SO_2, but it is not expected to seriously and adversely affect the results of this study. Sample size is rather small (n=7). Exposure conditions were not well defined – would not be reproducible. Data confirms previous reports that the nose is more efficient than the mouth at removing SO_2 – nothing new here.

Review – Confidence index ranking:

Low to moderate

Author: Frank, N.R., Speizer, F.E.

Internal ID: 170

Title: SO_2 Effects on the Respiratory System in Dogs

Year: 1965

Abstract: Mongrel dogs lightly anesthetized with pentothal were exposed to SO_2 by nose, by tracheal cannula, and by an isolated segment of trachea. Concentrations ranged from 7-230 ppm; exposure usually lasted 15-20 minutes. Among the animals breathing by nose, the changes in flow resistance at the nasal, laryngeal, and tracheoplumonary levels during exposure to and recovery from SO_2 may be characterized as follows: Nasal flow resistance ((Rn) increased roughly in proportion to the concentration of gas; this change occurred despite a slight average reduction in flow rate and became progressively greater with time in response to 25-61 ppm of SO_2. During recovery, which lasted 15 to 40 minutes, Rn reverted partially or completely to control levels. Pulmonary flow resistance (Rl) underwent fewer and relatively smaller changes than Rn during exposure; it rose and fell with about equal frequency. However, during recovery from SO_2, Rl tended to rise. The changes in laryngeal flow resistance (Rlx) –these data were fewest- approximated those of the lungs. In the second set of experiments in which SO_2 was administered by tracheal cannula, Rl usually rose quickly reaching a peak within several minutes; thereafter, it receded. Exposure of this type evoked the greatest change in Rl seen in the study. Limiting the area of exposure to the larynx and upper trachea produced a less pronounced increase in Rl. The pressure-gradient across the upper laryngotracheal segment, for a constant rate of flow, increased upon direct exposure to the segment to SO_2; a less consistent response was elicited when only the lower airways were exposed. None of the patterns referred to were invariable.

Objective: The purpose of this study was to compare the functional response of several levels of the respiratory system to the nasal inhalation of SO_2, an irritant gas.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
Study 1: SO_2 administered by nose 7-16 ppm 25-34 ppm 60-61ppm	Study 1: 20 minutes (but in a few instances exposures were purposely prolonged for 30-60 minutes)	Study 1: Dogs	Study 1: mongrel	Study 1: 1-3 or 4 years	Study 1: No info	Study 1: 8	No info	N/A	N/A
Study 2:SO_2 administered directly to the trachea		dogs	mongrel		No info	Study 2: 7			

60-215 ppm SO_2 and 60-230ppm in one dog									

Observations:

Among the animals breathing by nose, the changes in flow resistance at the nasal, laryngeal, and tracheoplumonary levels during exposure to and recovery from SO_2 may be characterized as follows: Nasal flow resistance (Rn) increased roughly in proportion to the concentration of gas; this change occurred despite a slight average reduction in flow rate and became progressively greater with time in response to 25-61 ppm of SO_2. During recovery, which lasted 15 to 40 minutes, Rn reverted partially or completely to control levels. Pulmonary flow resistance (Rl) underwent fewer and relatively smaller changes than Rn during exposure; it rose and fell with about equal frequency. However, during recovery from SO_2, Rl tended to rise. The pressure-gradient across the upper laryngotracheal segment, for a constant rate of flow, increased upon direct exposure to the segment to SO_2; a less consistent response was elicited when only the lower airways were exposed.

*Statistically significant

Author's Conclusions:

The changes in laryngeal flow resistance (Rlx) –these data were fewest- approximated those of the lungs. In the second set of experiments in which SO_2 was administered by tracheal cannula, Rl usually rose quickly reaching a peak within several minutes; thereafter, it receded. Exposure of this type evoked the greatest change in Rl seen in the study. Limiting the area of exposure to the larynx and upper trachea produced a less pronounced increase in Rl. The pressure-gradient across the upper laryngotracheal segment, for a constant rate of flow, increased upon direct exposure to the segment to SO_2; a less consistent response was elicited when only the lower airways were exposed.

Review – Assessment:

A. Test animals:	(+/-)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* (-) Are animal characteristics adequately recorded and appropriate for the objective of the study? a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* b) Age *Guideline: Adult, i.e.,8-12 weeks.* c) Sex d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* e) Weight variation *Guideline: Below 20% variation*

	f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (-)Was the duration of exposure appropriate to investigate the primary objective? (-)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure,*

	food should be withheld and water *May be withheld optionally.*
D. Equipment:	(-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? (-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random Assignment) (+) Was a control group assayed? (-) Is the control regime appropriate? (-)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined? (-)Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (-) Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (+)Was individual data reported for each test animal?
G. Data analysis:	(+/-) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+/-) Did the investigator appropriately interpret the statistics?

	(+/-) Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (-)Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Eight dogs were exposed to increasing concentrations of SO_2 (7-230 ppm) in order to determine which area of the upper respiratory tract was most responsible for removing irritant gases from the incoming air stream. In the dogs exposed by nose, there were changes in flow resistance at the nasal, laryngeal and tracheopulmonary levels during exposure to and recovery from SO_2. The exposure of SO_2 directly to the trachea, the changes in resistance were greater. There are few statistically significant findings in this study and it is difficult to discern any clinical significance due to problems in the study design listed below.

Confidence index:

The literature review is inadequate. Furthermore, lack of information as to animals source, gender, few numbers of animals actually exposed to the lower concentrations render statistical analysis moot.

STUDY 1:

There is no justification for the use of dogs as the test animals. There is no random assignment to exposure groups. Furthermore, the duration of exposure and the exposure levels differ from dog to dog. In addition, the number of control measurements varied between dogs. Most of the animals were reexposed to SO_2 (four exposures at the maximum) and the recovery period between exposures was only approximately 20 minutes.

The weight variation in the dogs was greater than 20% and the ages ranged from 1-3 or 4 years. The drugs used to anaesthetize may have affected lung function. Comparisons are made between these results and human studies. The statistical analysis was not based on all of the results. Only one control period and one exposure period picked randomly from each animal were chosen.

STUDY 2:

The sample size was too small. Not all of the dogs were treated similarly. For example, 2 were paralyzed, 4 had direct exposures to the trachea and 1 was exposed to 60-230ppm instead of 60-215 ppm. The results in study 1 and 2 are compared but the exposure levels and concentrations differed. There were no control animals.

Review – Confidence index ranking:

Low to moderate

Author: Azoulay-Dupuis, E., Bouley, G., Blayo, M.C.	**Internal ID:** 172
Title: Effects of Sulfur Dioxide on Resistance to Bacterial Infection in Mice	
Year: 1982	
Abstract: Continuous exposure to approximately a 10-ppm concentration of sulfur dioxide for periods of up to 3 weeks reduced the resistance of female mice to infection by aerosol inoculation with *Klebsiella pneumoniae*. The mortality rate rose and survival time shortened in SO_2-exposed animals compared to controls. Insofar as these results can be extrapolated to humans, the SO_2 concentrations used in this work is only found on certain industrial premises.	
Objective: The purpose of the present study was to determine the effects of continuous exposure to a high concentration of SO_2 (10ppm) on the resistance of mice to respiratory infection by aerosolized *Klebsiella pneumoniae* by monitoring their mortality rate and survival time.	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
10 ppm SO_2	1. 24 hrs 2. 1 wk 3. 2 wk 4. 3 wks	Mice	Pathogen free (SPF) OF_1	unknown	female	768 total (n=64 per experiment; each experiment performed in triplicate and there were 4 experiments)	Pathogen free	unknown	unknown

Observations:

A significant rise in mortality was observed in the groups exposed to SO_2 for periods of 7-21 days compared to their corresponding controls. A significant drop in mean survival time was observed from the 7th day of SO_2 exposure until the 21st day. Regardless of the duration of exposure, the decrease in mean survival time value was similar.
*Statistically significant

Author's Conclusions:

These experiments demonstrate that 10ppm of SO_2 reduces the resistance of mice to bacterial infection.

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+)c) Sex (No information on pregnancy) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective?

	(-)Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water May be withheld optionally.*
D. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+/-)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+) Were test animals randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random Assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined?

	(+)Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-)Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (+)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The continuous exposure to 10ppm SO_2 reduced the resistance of mice to infection. After 1 week of continuous exposure to SO_2, mice exhibited a significant increase in mortality and a decrease in survival time. However, the rate of increase immortality did not rise as exposure time lengthened. The concentration of 10 ppm is above that expected in the ambient air. However, the data may apply to continuous exposure over time in an occupational setting. In addition, results demonstrate that not only does chronic exposure directly affect the respiratory system it can also have an affect on the immune system. This could have important implications on sensitive populations such as immune-compromised individuals. The results suggest the need for more basic studies of the interaction of SO_2 and virus or bacterial strains with the cell.

Confidence index:
The study was very well designed. There were a total of 768 animals used for the experiments. Each experiment consisted of 32 exposed animals and 32 controls. Also, each experiment was performed in triplicate. There were several different exposure times tested. The appropriate dose of bacteria was determined from preliminary tests. There is consistency reported in other studies. The method and results section are very detailed. In addition, because of the well defined exposure conditions this study should be easily reproducible. For example, the study included a series of control groups and performed a time course of exposure. Only one endpoint was studied – mortality. The study yielded novel results not only because 10 ppm can reduce resistance to bacterial infection, but also because there was no increase in mortality with increased exposure time.

However, the acclimation period used for this study is not specified and only one exposure level is used. Furthermore, there is no information on the age and diet of the mice and there is limited information on the monitoring devices.

Review – Confidence index ranking:

High to moderate

Author: Grose, E.C., Grady, M.A., Illing, J.W., Daniels, M.J., Selgrade, M.K., Hatch, G.W.

Internal ID: 174

Title: Inhalation Studies of Mt. St. Helens Volcanic Ash in Animals III. Host Defense Mechanisms

Year: 1986

Abstract: The effects of inhalation exposure of mice or rats to 9.4 mg/m3 volcanic ash, 2.5 mg/m3 SO_2, or both on host defense mechanisms were assessed. Cytologic changes in pulmonary lavage fluid included an increase in percentage polymorphonuclear leukocytes due to SO_2 exposure and an increase in eosinophils due to ash. SO_2 and ash also produced decreases in percentage alveolar macrophages. In the case of ash-exposed animals, this decrease was offset by an increase in lymphocytes. Total cell counts and viability were not affected by any of the exposures. Pulmonary clearance mechanisms were affected in that there were both decreased alveolar macrophage phagocytic capability following ash and ash + SO_2 exposures and depressed ciliary beat frequency attributable to ash exposure. None of the inhalation exposures caused increases in susceptibility to an immediate or 24 hr postexposure aerosol challenge with Streptococcus. However, intratracheal instillation of both fine- and coarse-mode volcanic ash caused slight but significant increases in mortality due to bacterial challenge 24 hr after the instillation. The phytohemagglutinin-induced blastogenic response of splenic lymphocytes from exposed animals did not differ significantly from that of control lymphocytes, although the lipopolysaccharide-induced blastogenic response was enhanced. Ash exposure had no effect on susceptibility to murine cytomegalovirus. In summary, volcanic ash alone or in combination with SO_2 had only minimal effects on certain host defense mechanisms.

Objective: The present study employs assays of different aspects of host defense and applies these methods to determine effects of Mt. St. Helens volcanic ash. Few studies have been performed on potential effects of this ash on respiratory host defense.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0.95±0.05ppm SO_2 1. SO_2 alone 2. SO_2 + ash	1. 2hr (acute) 2. 2hr (acute) + 24 hr recovery 3. (intermittent) 2hr/day, 5 days	Mice Rats	CD-1 Sprague-Dawley CD	4-8 wks 60-70 days	Female male	160	No info	unknown	unknown

Observations:

Inhalation exposure of mice to SO_2 or ash+SO_2 followed by a bacterial aerosol challenge either immediately after ash exposure or 24 hr postexposure caused no significant change in the incidence of mortality caused by infection. Exposure to SO_2 increased ($P<0.01$) the percentage polymorphonuclear leukocytes (PMN) and decreased ($P<.01$) the percentage alveolar macrophages (AM). Although significant alterations in cell types occurred, there was not a significant change in either total cell numbers or total cell viability 0 or 24 hrs following exposure. The combination of ash+SO_2 caused decreased phagocytosis ($P<0.05$) when

compared with the other treatment groups. At 24 hr postexposure, phagocytosis was depressed ($P<0.05$) after exposure to SO_2, alone or in combination with ash. Changes in the effect of a treatment over time showed that there was a decrease in phagocytosis in the 24-hr SO_2 group that was significantly ($P<0.01$) less than occurred at 0 hr. In addition, intratracheal instillation of both fine- and coarse-mode volcanic ash caused slight but significant increases in mortality due to bacterial challenge 24 hr after the instillation. The phytohemagglutinin-induced blastogenic response of splenic lymphocytes from exposed animals did not differ significantly from that of control lymphocytes, although the lipopolysaccharide-induced blastogenic response was enhanced. Ash exposure had no effect on susceptibility to murine cytomegalovirus.
*Statistically significant

Author's Conclusions:

"Acute exposure to SO_2 at the concentrations used in this study appeared to cause minimal effects on pulmonary and systemic defense mechanisms."

Review – Assessment:

A. Test animals:	(+)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of

	appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (+/-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (+)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water May be withheld optionally.*
D. Equipment:	(-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?

E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (+/-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random Assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+/-)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined? (-)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (+)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding

	variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Acute exposure to volcanic ash at the concentrations used in this study appeared to cause minimal effects on pulmonary and systemic defense mechanisms. Susceptibility to bacterial infection was slightly increased following intratracheal injection of ash, but not after an inhalation exposure. The conclusions of the study focus on the effects of the volcanic ash, not SO_2. Furthermore, This study was not designed to test the effect of varying levels of SO_2 on host defense mechanisms to viral infection, therefore it does not support human exposure guidelines.

Confidence index:

Exposure conditions and exposure assessment were performed using existing, established protocols cited in the literature. Assessment was quite thorough with multiple assays performed to study various aspects of host defense – looked at systemic effects, acute inflammation, upper airway clearance, activity of alveolar macrophages, host susceptibility to pulmonary infection in response to SO_2 exposure. What was unclear about this study was the exposure conditions cited in methodology were not adequately described. In addition, the study dealt mainly with the effect of ash exposure and there is limited discussion regarding the SO_2 exposure. Furthermore, there was no information on the weight or pretrial health of the mice or rats. In addition, for the rat study, there is no information on the number of rats used.

Review – Confidence index ranking:

Moderate

Author: Atson, L, Bannenberg, G., Corriga, A., Lou, Y., Lundberge, G., Ryrfeldt, A. Moldeus, P.,

Internal ID:178

Title: Sulfur Dioxide-Induced Bronchoconstriction via Ruthenium Red-Sensitive Activation of Sensory Nerves

Year: 1991

Abstract: The mechanism of sulfur dioxide-induced bronchoconstriction was studied using isolated perfused and ventilated guinea-pig lungs. They were exposed to sulfur dioxide after pretreatment with different compounds, either via the pulmonary artery or via the air passages. Neither the cyclooxygenase inhibitor indomethacin (30uM) nor the H1-receptor antagonist diphenhydramine (15 uM), given via the perfusate, attenuated the sulfur dioxide-induced bronchoconstriction. Furthermore, sulfur dioxide exposure did not cause a release of either thromboxane or histamine into the perfusate. In experiments with atropine equivocal results were obtained with regard to protection against sulfur dioxide-evoked bronchoconstriction. Intratracheal instillation of the local anesthetic agent lidocaine (1mg/50ul) markedly reduced the sulfur dioxide-induced bronchoconstriction. Also, ruthenium red (10uM), an agent with calcium entry-blocking properties and an inhibitor of capsaicin-induced bronchoconstriction, was able to inhibit the effect of sulfur dioxide. The sulfur dioxide-induced bronchoconstriction was associated with release of calcitonine gene-related peptide, a sensory neuropeptide. The effect of sulfur dioxide was also inhibited by a Ca^{2+}-free buffer plus EGTA. These results suggest that sulfur dioxide-induced bronchoconstriction in the guinea-pig lung is the result of a local effect on sensory nerves (C-fiber activation). The mechanism seems to be dependent of the Ca^{2+}-dependent release of sensory neuropeptides and to be linked to opening of the cation channel, which is associated with the proposed capsaicin receptor on sensory nerves as revealed by the inhibitory effect of ruthenium red.

Objective: To study the mechanism of sulfur dioxide-induced bronchoconstriction using isolated perfused and ventilated guinea-pig lungs.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
250 ppm	10 minutes	Guinea pigs	Dunckin-Hartley	NA	male	Not clearly stated; for each experiment n=3 to n=6	NA	No observed adverse effect level	Lowest observed adverse effect level

Observations:

Neither the cyclooxygenase inhibitor indomethacin (30uM) nor the H1-receptor antagonist diphenhydramine (15 uM), given via the perfusate, attenuated the sulfur dioxide-induced bronchoconstriction. Furthermore, sulfur dioxide exposure did not cause a release of either thromboxane or histamine into the perfusate. In experiments with atropine equivocal results were obtained with regard to protection against sulfur dioxide-evoked bronchoconstriction. Intratracheal instillation of the local anesthetic agent lidocaine (1mg/50ul) markedly reduced the sulfur dioxide-induced bronchoconstriction. Also, ruthenium red (10uM), an agent with calcium entry-blocking properties and an inhibitor of capsaicin-induced bronchoconstriction, was able to inhibit the effect of sulfur dioxide. The sulfur dioxide-induced bronchoconstriction was associated with release of calcitonine gene-related peptide, a sensory neuropeptide. The effect of sulfur dioxide

was also inhibited by a Ca^{2+}-free buffer plus EGTA.
*Statistically significant

Author's Conclusions:

SO$_2$ induced bronchoconstriction does not appear to be due to the release of bronchoreactive agents such as thromboxane or histamine since neither cyclooxygenase inhibition or histamine antagonist provided protection. Instead this model suggests that stimulation of some local neuronal reflex arch is the cause. This is indicated by the protective effect of lidocaine and the release of the peptide CGRP-Li. The effect also appears to be calcium dependent and the inhibition obtained by ruthenium red indicates a possible neuropeptide release involvement.

Review – Assessment:

A. Test animals:	(-) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+/-) Was the duration of exposure appropriate to investigate the primary

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C. Housing/Feeding:	N/A Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* N/A Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* N/A Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* N/A Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* N/A Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+/-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days*

	(-) Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (+/-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+/-) Was a control group assayed? (+/-) Is the control regime appropriate? (+/-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense?

	. (+) Are the extrapolations reasonable? . (+) Is the paper in a peer-reviewed journal? . (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Several mechanisms may be involved in SO_2-induced bronchoconstriction. In this study, the findings indicated that SO_2 or its products did not react directly with critical cell(s) in the lung that may lead to the release of bronchoactvie agents such as thromboxane or histamine. The investigators also determined that SO_2 does not trigger the bronchoconstriction via a neuronal parasympathetic reflex pathway. The role of an involvement of the cholinergic system remained unclear. The effect appears to be Ca^{2+}-dependent and a neuropeptide release is possibly involved. This study gives further evidence to the mechanism of SO_2 induced bronchoconstriction but there are problems with the study design that limit the usefulness of the findings. An undetermined number of guinea pigs were exposed to 250 ppm SO_2 and various neurochemical blockers and inhibitors in an attempt to ascertain exactly how SO_2 may cause bronchoconstriction. Since only one concentration of SO_2 was used, this study has limited value to human exposure guidelines.

Confidence index:

The number of animals used is not clearly stated. The experiments ranges from n=3 to n=6. The sample sizes are too small for results to be considered statistically significant. The weight variation in the rats is greater than 20%. There is no information on the age or pretrial health of the animals. The animals were not randomly assigned to exposure groups and it is not clear if there was a control group assayed. Furthermore, the exposure duration is not clearly defined. The results from this study are difficult to compare with other studies as the breathing was not natural. Instead, the lungs were connected to breathing air. None of the results are statistically significant. Lack of information as to number of animals, prior health, diet, exposure times detract from an otherwise good study.

Review – Confidence index ranking:

Moderate

Author: Barry DH, Mawdesley-Thomas LE	**Internal ID: 181**
Title: Effect of sulfur dioxide on the enzyme activity of the alveolar macrophage of rats	
Year: 1970	
Abstract: Adult rats were exposed to sulfur dioxide to induce hypersecretion of mucus and so increase the amount of mucus reaching the alveoli. The comparative activity of four lysosomal hydrolytic enzymes was studied histochemically in free alveolar cells, thought to be macrophages, from the lungs of the exposed and control animals. Although the activity of β-glucuronidase, β-galactosidase, and N-actyle-β-glucosaminidase appeared increased in some free alveolar cells in the peribronchiolar region, a marked increase of acid phosphatase activity was seen in the free alveolar cells of the lung parenchyma. It is suggested that in rats acid phosphatase in alveolar macrophages is connected with the catabolism and removal of mucopolysaccharide and increases in response to the extra amount of mucus reaching the alveoli after respiratory irritation with sulfur dioxide.	
Objective: To compare the activity of several lysosomal hydrolytic enzymes in the lungs of rats exposed to SO_2 or unexposed rats.	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
300ppm	**6 hours/day for 10 days**	**rat**	**Carworth Europe**	**n/a 200grams**	**12M 12F**	**24**	**n/a**	**n/a**	**300ppm**

Observations:

"Enzyme activity of the four systems studied was observed in free alveolar cells from the control animals from the control animals although β-galactosidase and N-acetyl-β-glucosaminidase appeared weakly active. All the free alveolar cells from the treated animals showed an increased activity of acid phosphatase, but the other three enzyme systems appeared increased only in some free cells adjacent to bronchi."
*Statistically significant

Author's Conclusions:

"The effects of irritation by sulfur dioxide on the lungs of rats probably consisted of hypersecretion of mucus with a corresponding increase in macrophage hydrolase activity, of which only acid phosphatase activity was large enough to be demonstrated histochemically. The hypersecretion of mucus is not specific for this irritant and it is probable that other gaseous irritants and probably particulate irritants may well produce the same sequence of events."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie

	☐ *Guideline: Rat, or justification for use of another mammalian specie.* +b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* +f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -+✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ±-✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	-✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*

D. Equipment:	-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* -✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? ±✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? ±✎ Were assessment measures sufficiently sensitive? +/-✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	-✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? ±✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? -✎ Are the results novel? ±✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +-✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal?

	Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

24 rats equally representing male/female were exposed to 300 ppm SO_2 for 6 hrs/day/10 days to induce hypersecretion of mucus. This study was not specifically examining the effects of increasing gradients of SO_2 on the lungs, rather the objective was to determine what happens to mucus that arrives in the lung, and what enzymes were activated. As such this study has little value to human exposure guidelines.

Confidence index:

The means of exposure and data collection are poorly described, and no raw data or statistical analyses were provided. Endpoint chosen is histochemical analysis of enzyme activity, however, data is not even shown for histochemical analysis of β-glucoronidase, β-galactosidase, and N-acetyl-β-glucosaminidase activity in control versus SO_2 treated rats making it hard to have any confidence in the authors conclusion that there were no changes in the activity of these three enzymes. As such, the reader cannot have a great deal of confidence in the study. Lack of information as to animal health (pre and during experiment), diet, reagent source, monitoring equipment, lack of any reported statistical analysis all limit confidence in this study.

Review – Confidence index ranking:

Low to moderate

Author: Fairchild, G. A., Roan, J.,McCarroll, J. **Internal ID:** 182

Title: Atmospheric Pollutants and the Pathogenesis of Viral Respiratory Infection.

Year: 1972

Abstract: Influenza-infected mice exposed continuously to 20 ppm of sulfur dioxide (SO_2) for seven days after virus exposure developed more pneumonia than virus control mice. Dose-response experiments suggested that the post virus SO_2-induced increase in influenzal pneumonia began at about 7 to 10 ppm. The increase in pneumonia was not influenced by altering the virus dosage within a 5 to 100 mouse infectious dose range. A similar increase (15% to 20%) in influenzal pneumonia was observed when mice were exposed to 25 ppm of SO_2 prior to initiation of virus infection. The SO_2 exposure had no effect on the growth of influenza virus in the lungs of mice. Rather, the increase in the amount of pneumonia was associated with SO_2 concentrations that induced low-grade, inflammatory lesions in the lung.

Objective:
To assess the effect of SO_2 alone on the pathogenesis of murine influenza.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
3.4 – 34.5 ppm	Up to 7 days	mice	SPF Swiss Webster albino	7-8 weeks	male	unknown	unknown	unknown	7-10 ppm

Observations:

The Effect of SO_2 Exposure on the Pathogenesis of Influenza Pneumonia
At 19.3 ppm and higher, significantly increased amounts of pneumonia were observed.

The Effect of Virus Dosage on Amount of Pneumonia in SO_2 Exposed Mice
There was no greater difference in the amount of pneumonia between virus-SO_2 exposed groups and their respective virus control group as a function of virus dosage within the 5 to 100 MID_{50} dose range used in these experiments.

The effects of exposure to SO_2 prior to virus exposure on the development of pneumonia in influenza infection
A significant increase in the amount of pneumonia occurred only at the 25 ppm concentration after four to seven days' continuous exposure. The magnitude of the increase in the amount of pneumonia (16.7%) was similar to that seen in the preceding experiments where SO_2 exposure was preceded by initiation of virus infection.

Histapathological Effects of SO_2 on the Lung

Histapathological effects were observed at 27 ppm and higher after 7 days' exposure.
*Statistically significant

Author's Conclusions:

The amount of pneumonia that developed following influenza infection in the mouse was altered as a result of inhalation of SO_2. This effect is dependent on the exposure dose and is characterized by a dichotomous response. At the lower SO_2 concentrations (2.9-5.3 ppm) a slightly reduced amount of pneumonia was observed on the seventh day after initiation of infection. At concentrations of 19.3 ppm and higher, the amount of pneumonia was obviously increased.

The concentration of SO_2 required to increase influenzal pneumonia in mice was approximately 100 times greater than that concentration commonly observed in New York. This and other evidence concerning the toxic effect of SO_2 on the respiratory system suggests that this injury is not responsible for the episodes of increased mortality and other respiratory illnesses which have been associated with elevated SO_2 levels.

Review – Assessment:

A. Test animals:	(-) Is the number of animals sufficient to produce statistically significant results? + *Guideline: 5-animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex (+) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (+) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? + *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (-) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period?

	Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates. (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration,

	reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study's aim was to determine what affect, if any, SO_2 exposure would have the ability of influenza virus to infect mice. Influenza-infected mice exposed continuously to 20 ppm of sulfur dioxide (SO_2) for seven days after virus exposure developed more pneumonia than virus control mice. The SO_2 exposure had no effect on the growth of influenza virus in the lungs of mice. Rather, the increase in the amount of pneumonia was associated with SO_2 concentrations which induced low-grade, inflammatory lesions in the lung. However, the poor reporting and awkward format of the report inhibits the interpretation of the results. This study was also not specific to just SO_2 affect on the respiratory system of the mice, but attempted to determine the correlation between the SO_2 concentration and the immune response. This correlation seems tenuous and of little value to human exposure guidelines. Furthermore, it is difficult to interpret the results as it is unclear as to how many animals are used in each experiment.

Confidence index:

Exposure conditions and assessment methods were well defined; therefore the study should be easily reproducible. However, There was no clear information on the sample sizes.

Sufficient range of exposure conditions was used to investigate the effects of SO_2 on pathogenesis of viral respiratory infection. Single endpoint measured – development of influenzal pneumonia in mice. However, no reasonable explanation for why at lower concentrations of SO_2, a slightly reduced amount of pneumonia was observed compared to untreated controls – warrants further investigation. Furthermore, the results of this experiment were combined with earlier single factor experiments that were said to have similar results. But, there is no information on these earlier experiments provided.

Review – Confidence index ranking:

Moderate

Author: Fedde MR, Kuhlmann WD | **Internal ID: 183**

Title: Cardiopulmonary responses to inhaled sulfur dioxide in the chicken

Year: 1979

Abstract: Selected cardiopulmonary variables were measured in anesthetized, male, White Leghorn type chickens before, during, and after 60min exposure to various inhaled SO_2 concentrations ranging from 0 to 5000 ppm. In one series of experiments, birds breathed through their nostrils and mouth; in a second series they breathed through a tracheal cannula. Exposure to 5000 ppm SO2 rapidly caused death in all brids with cannulated tracheae and in 4 of 5 birds with an intact respiratory system; one bird in each series of experiments died when exposed to 1000 ppm. Inhaling 100 ppm SO_2 did not alter heart rate, blood pressure, tidal volume, respiratory frequency, or arterial blood gases and pH. Airway resistance was decreased by inhaling 500 ppm SO_2 and was initially decreased but later greatly increased by inhaling 1000 ppm. Respiratory frequency and minute volume were decreased and $PaCO_2$ was increased by inhaling 1000 ppm. The cardiopulmonary responses to inhaling 5000 ppm were generally augmentations of those responses, which occurred when 1000 ppm was inhaled. The SO_2 could not be detected in caudal thoracic or abdominal air sac gas at any concentration inhaled, but vascular congestion and areas of brown discoloration appeared in the lungs of all birds that inhaled 5000ppm and some that inhaled 1000 ppm. Mucous secretion in the trachea increased in some birds that inhaled 500ppm SO_2 and in most that inhaled higher concentrations. Chickens appear to have more tolerance for inhaled SO_2 than most mammals but the pollutant gas at high concentrations detrimentally affects the cardiopulmonary system.

Objective: "This study was to determine effects of several concentrations of inhaled SO2 on various cardiopulmonary variables in spontaneously breathing chickens."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0-5000ppm	**60min**	**Chicken**	**White Leghorn**	**Adult**	**male**	**54**	**n/a presumed healthy**	**0ppm**	**1ppm**

Observations:

Lethal exposures to SO_2 occurred after 1000ppm and 5000ppm SO_2, but no deaths were reported after 100 or 500ppm exposures, for both naturally breathing and tracheally cannulated birds.

In normal breathing birds:

At 100ppm, a small but statistically significant increase in minute volume was noted.

At1000ppm, "…air sac pressure and airway resistance significantly decreased for about 15min, then greatly increased for the remainder of the exposure period… Arterial pH and $P0_2$ were significantly decreased, while $PaCO_2$ increased, indicating hypoventilation and retention of CO_2.

At 5000ppm, the effects seen at 1000ppm were further exacerbated: "..5000ppm concentration significantly increased heart rate and tidal volume and significantly decreased respiratory frequency and minute volume. Blood gas and pH changes were greater than in those birds receiving 1000ppm SO2 but significant changes were not produced in blood pressure or HCO_3^- in the arterial blood.

In birds with tracheal cannulae:

"Exposing birds with cannulated tracheae to lower concentrations of SO_2 produced effects similar to those in birds with intact respiratory systems. The predominant effects were on minute volume, airway resistance, arterial pH, and PCO_2."

Necropsy findings:

No gross pathology was seen in birds exposed to 0 or 100ppm SO_2 in either normal or cannulated birds. Birds exposed to 1000ppm, however, had excess mucus in the trachea and syrinx, though lungs appeared normal. Both upper airways and lungs in birds receiving 5000ppm SO_2 showed gross pathological changes. These included large amounts of mucus in the trachea, vascular congestion, and brown mucosa of the primary bronchus, suggesting fixation of the tissues by SO_2.

*Statistically significant

Author's Conclusions:

"These experiments clearly showed that SO_2 harmed the cardiopulmonary system of the chicken… The most prominent effect of SO_2 on the chicken was on airway resistance. The initial decrease in resistance was followed by an increase as exposure to higher concentrations continued… Gross lung pathology occurred only in birds with an intact trachea only at the highest concentrations (5000ppm) of SO_2... in a few birds with cannulated tracheae, 1000ppm SO_2 also caused lung lesions visible on gross examination."

Review – Assessment:

A. Test animals:	+✏ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✏ Are animal characteristics adequately recorded and appropriate for the objective of the study? ±a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* +b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✏ Were the chosen exposure levels appropriate to investigate the primary objective? -✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? +✏ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.*

	+✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? ±✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* ±✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	+/-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? +✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? ±✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +-✎ Was the nature and severity of symptoms graded against an explicitly defined scale?

	+✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? +-✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✏ Was individual data reported for each test animal?
G. Data analysis:	+✏ Are the statistical methods used appropriate? +✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective? +✏ Were assumptions reasonable? +✏ Are the methods reproducible? +✏ Did either the study design or the statistical analysis limit confounding variables? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

SO_2 harmed the cardiopulmonary system of the chicken but the animals did not have as sensitive response as most mammals. Airway resistance decreased then increased as exposure to higher concentrations continued. Respiratory frequency and minute volume were significantly reduced by inhaling 1000 ppm SO_2, tidal volume was not significantly altered. The importance of the upper airway in removing SO_2 was indicated by the fact that gross lung pathology occurred in birds with an intact trachea only at 5000 ppm. This study is valuable to human exposure guidelines because it does lend value to weight of evidence for exposure levels, the fact that a range of levels were provided, and several different types of measurements were made. Also, the efficiency of the upper respiratory tract in protecting the lower respiratory tract from damage.

Confidence index:

The sample size (n=54) and experimental methods appear to be sufficient to produce results of good quality. Minor information deficits such as the source, feed and health of the chickens pre exposure, reported sensitivity of the detection instruments, and how the animals were assigned to control groups detracts somewhat from confidence in the study. Exposure conditions and assessment methods were well defined – should be easily reproducible. Did attempt to limit confounding variables by using same sex, age animals. Also made comparison of cardiopulmonary variables to preexposure values for each bird, eliminating much inter-bird variation. Results are in agreement with other published works in geese in terms of decrease in airway resistance following acute exposure to high levels of SO_2.

Review – Confidence index ranking:

High to moderate

Author: Grunstein, M. M. Hazucha, M. Sorli, M. H. Milic-Emili, J.	**Internal ID: 186**
Title: Effect of SO_2 on control of breathing in anesthetized cats	
Year: 1977	
Abstract: In seven anesthetized tracheotomized cats we studied the acute respiratory effects of SO_2 inhalation at different steady-state levels of arterial CO_2 tension ($Paco_2$). During room air breathing, SO_2 (0.05%) addition caused a progressive reduction in tidal volume (V_T) and increases in both respiratory frequency (f) and pulmonary resistance (R_L). Atropine sulfate abolished the bronchoconstriction response to SO_2 and thus permitted the study of the influence of SO_2 on V_T and f in the absence of constricted airways. Despite marked reductions in the V_T VS. $PaCO_2$ relationships with SO_2 exposure after atropine, the relationship between pulmonary ventilation (VE) and $PaCO_2$ was not significantly altered. This was the case since SO_2 caused solely a reduction in inspiratory duration (T_i), affecting neither the mean rate of rise of inspiratory activity (i.e., V_T/T_i) nor the relationship between T_i and breath duration. Thus, airways irritation with SO_2 produced rapid, shallow breathing characterized by a shortening of inspiratory and total respiratory cycle times with no change in the rate of development of inspiratory activity. The findings suggest an influence exclusively concerned with the timing of inspiration. Perhaps premature onset of inspiratory activity accounts for the observed effects.	
Objective: To evaluate the acute respiratory effects of SO_2 exposure before and after administration of atropine sulfate (a known bronchodilator) to help identify which mechanisms regulating the depth and frequency of breathing are affected during irritant gas inhalation.	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
.03-.07%	20-40 sec	cat	unknown	adult	unknown	7	unknown	unknown	.05%

Observations:

During room air breathing, SO_2 (0.05%) addition caused a progressive reduction in tidal volume (VT) and increases in both respiratory frequency (f) and pulmonary resistance (R_L). Atropine sulfate abolished the bronchoconstriction response to SO_2 and thus permitted the study of the influence of SO_2 on V_T and f in the absence of constricted airways. Despite marked reductions in the V_T vs. $PaCO_2$ relationships with SO_2 exposure after atropine, the relationship between pulmonary ventilation (V_E) and $PaCO_2$ was not significantly altered. This was the case since SO_2 caused solely a reduction in inspiratory duration (T_i), affecting neither the mean rate of rise of inspiratory activity (i.e., V_T/T_i) nor the relationship between T_i and breath duration. Thus, airways irritation with SO_2 produced rapid, shallow breathing characterized by a shortening of inspiratory and total respiratory cycle times with no change in the rate of development of inspiratory activity.
*Statistically significant

Author's Conclusions:

The present results have shown that, under constant chemical drive conditions, the postatropine respiratory response to SO_2 in characterized by a number of features, including a decrease in V_T, shortening of T_i and T_{tot}, a constancy in the rate of rise of inspiratory activity, and in some instances an increase in FRC. This response suggests that the vagi potentiate the inspiratory on-switch. This is supported by the observation that during maintained lung inflation, the duration of apnea was found to be markedly reduced by SO_2. Such an inspiratory-excitatory effect has been implicated in a number of other studies where lung irritant receptors were stimulated.

Review – Assessment:

A. Test animals:	(-) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+/-) c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (-) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (-) Was the duration of exposure precisely defined? N/A If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	N/A Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test

	and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* N/A Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* N/A Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water* *may be withheld optionally.*
D. Equipment:	N/A Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* N/A Was a full description of the monitoring devices provided? N/A What was the sensitivity of the monitoring devices employed?
E. Procedural:	(+/-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (+/-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+/-) Was a control group assayed? (+/-) Is the control regime appropriate? (-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams?

	(+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study evaluated the effects of SO_2 exposure before and after administration of atropine sulfate, an agent known to inhibit the bronchoconstrictor response to SO_2. Airways irritation with SO_2 produced rapid, shallow breathing characterized by a shortening of inspiratory and total respiratory cycle times with no change in the rate of development of inspiratory activity. The findings suggest an influence exclusively concerned with the timing of inspiration. Perhaps premature onset of inspiratory activity accounts for the observed effects.

Confidence index:

Exposure conditions were not well defined – suddenly made to inhale from bags containing SO_2, may not be easily reproducible. Each cat served as its own internal control, no additional attempts were made to limit confounding factors: unknown sex, age, weight, and health. Only a single drug was used to abolish bronchoconstriction, should be tested with other drugs to see if similar respiratory effects are found. Study is in agreement with other published literature concerning cats, in direct contrast with studies in rabbits – which model best approximates humans is unclear. However, The sample size used in this study was too small. Preliminary studies were conducted on the 7 cats in two separate groups (n=3 and n=4). Furthermore, there was no justification for the use of cats and there was no information on the age or pretrial health of the cats.

Review – Confidence index ranking:

Low to moderate

Author: Eady, R.P., Jackson, D.M.	**Internal ID:** 190
Title: Effect of nedocromil Sodium on SO_2-Induced Airway Hyperresponsiveness and Citric Acid-Induced Cough in Dogs	
Year:1989	
Abstract: In anaesthetized dogs exposed to SO2, nedocromil sodium prevented the increase in bronchial responsiveness to histamine challenge and suppressed the cellular changes taking place in the lung. In conscious dogs challenged with citric acid, by inhalation, nedocromil sodium prolonged the time to onset of coughing and had an inhibitory effect on the total number of coughs in each episode. Nedocromil sodium has been observed to stimulate bronchial C fibers, and it is suggested that this activity may relate to the antitussive effect of the compound.	
Objective: To test the activity of nedocromil sodium, a new drug for the prophylactic treatment of asthma, using a SO_2 exposed dogs as a model of bronchial hyperresponsiveness (BHR).	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
400 ppm SO_2	2 hours	Dogs	beagle	No info	No info	8	No info	N/A	N/A

Observations:

Exposure to SO_2 caused an immediate increase in bronchial responsiveness to histamine, which returned to normal levels approximately 2 h after exposure. A second phase of BHR occurred at 24 h and lasted for several days. Nedocromil sodium given before and after SO_2 exposure prevented the immediate increase in lung responsiveness and reduced the persistent increase in BHR at 24 h. The drug did not affect the responsiveness of normal dogs to histamine. The total number of cells recovered by BAL was increased following SO_2 exposure.
*Statistically significant

Author's Conclusions:

"Nedocromil sodium has been observed to stimulate bronchial C fibres, and it is suggested that this activity may related to the antitussive effect of the compound."

Review – Assessment:

A. Test animals:	(-)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the

	objective of the study? (-)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+/-)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+)Was evidence provided to justify the dosing regime? (-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers

	appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water* *May be withheld optionally.*
D. Equipment:	(-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? (-) Were test animals randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random Assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive?

	(-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+/-)Did the investigator appropriately interpret the statistics? (+/-)Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (+)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

8 beagles were exposed to 400 ppm SO_2 for 2 hours in order to induce airway hyperresponsiveness. The goal of this study was not to measure the effect of SO_2 on dogs, rather it was to asses the effectiveness of nedocromil sodium to suppress cough in dogs. Therefore, this study does not provide support for human exposure guidelines.

Confidence index:

This study gives no justification for the use of dogs. Furthermore, there is very limited information provided for most sections of the study including: test animals, exposure conditions, housing/feeding/ , equipment and procedural and the sample size is too small to achieve statistically significant results. In addition, the animals are not randomly assigned to exposures.

This study gives limited information on statistical analyses and makes use of many references that were written by the same authors. In addition, there is no discussion on whether the anaesthetic (chloralose) affects lung function. Furthermore, the exposure conditions and assessment methods were not well defined – consequently the study would not be easily reproducible.

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM

Author: Baskurt, OK	**Internal ID:** 192

Title: Acute Hematologic and Hemorheologic Effects of Sulfur Dioxide Inhalation

Year: 1988

Abstract: Fifty male rats were exposed to 0.87 ppm sulfur dioxide (SO_2) for 24 hours. Hematologic and hemorheologic parameters measured in this group were compared with the results of a control group of 51 male rats. Hematocrit values were found to be higher ($p<.005$) in the SO_2-treated group (43.55 ± 0.41%, mean ± standard error), when compared to the control group value (41.97 ± 0.35%). Sulfhemoglobin values were also higher ($p < 0.0001$) in the SO_2-treated group (0.60 ± 0.08%) than the control group (0.08 ± 0.02%). Osmotic hemolysis ratio was slightly increased ($p<0.05$) in the 0.55% sodium chloride solution. However, whole blood and packed cell viscosities were lower in the SO_2-treated animals, while no significant differences were observed in the plasma viscosities. The mechanism of these effects could not be clarified completely, but structural and functional effects of SO_2 inhalation on peripheral erythrocytes were discussed.

Objective: To investigate the effect of SO_2 inhalation on blood, particularly on its rheologic properties. Previous studies have shown that hemoglobin structure may be altered due to air pollutants. Changes in hemoglobin structure and concentration is one of the major determinants of the rheologic character of red blood cells.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0.87 ppm	24hr	rats	Swiss albino	-	male	101	healthy	0 ppm	0.87 ppm

Observations:

Hematologic parameters

Hematocrit values in the SO_2-treated group were significantly higher than in the control group ($p<.005$).

The differences in erythrocyte counts and hemoglobin values were not significantly different between the experimental and control groups.

The MCV (mean corpuscular volume) and MCHbC (mean corpuscular hemoglobin concentration) values of the two groups were not statistically different.

Sulfhemoglobin ratios were significantly higher in the SO_2-treated group ($p<5x10^{-7}$).

The difference between methemoglobin ratios was not significant.

The osmotic hemolysis in the NaCl solutions at concentrations of 0.55%, 0.50J%, and 0.45% was enhanced in the SO_2-treated group. The difference between the hemolysis ratios in the 0.55% solution was significant ($p<0.05$)

Hemorheologic results

Plasma viscosities measured at 230sn^{-1} shear rate were not significantly different in control and experimentaly groups.

The differences in viscosities between the two groups ws significant only at 46 sn^{-1} shear rate ($p<0.05$).

Packed cell viscosities were significantlylower in the SO_2-treated group at all shear rates at which the measurements were done ($p<.01$), except the 23 sn^{-1} shear rate.

*Statistically significant

Author's Conclusions:

It has been shown that exposure of rats to .87ppm SO_2 for 24 h leads to an increase in hematocrit values. This may be related to the effects of SO_2 on the structure of peripheral erythrocytes, rather than to a relative or absolute increase in the red cell population, inasmuch as the erythrocyte counts did not change significantly. However, SO_2 itself may not be responsible for these effects, because it can be easily hydrated to yield sulfurous acid on the surfaces of the respiratory tract. Therefore, some other species of SO_2 may be expected to reach the blood during SO_2 inhalation.

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* (+) b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* (-) c) Sex (+/-) d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation ☐ *Guideline: Below 20% variation* (+/-) f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(+/-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period?

	Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates. (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+/-) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment)

	. (+) Was a control group assayed? . (+) Is the control regime appropriate? . (-) Was the period of observation following exposure appropriate? □*Guideline: Longer than 14 days.*
F. Data collection:	. (+) Were appropriate endpoints examined? . (+/-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? . (+) Were accepted assessment methods employed? . (+) Were assessment measures sufficiently sensitive? . (+) Was the nature and severity of symptoms graded against an explicitly defined scale? . (+) Did qualified personnel conduct the exams? . (+) Was the timing and frequency of assessment logical? . (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? . (-) Was individual data reported for each test animal?
G. Data analysis:	. (+) Are the statistical methods used appropriate? . (+) Are confidence intervals reported? . (+) Did the investigator appropriately interpret the statistics? . (+) Are the observed trends statistically significant?
H. Interpretations:	. (+) Was the original objective addressed? . (+/-) Are the results novel? . (+) Was the design of the study appropriate to investigate the primary objective? . (+) Were assumptions reasonable? . (+) Are the methods reproducible? . (+) Did either the study design or the statistical analysis limit confounding variables? . (+) Do the conclusions make biological sense? . (+) Are the extrapolations reasonable? . (+) Is the paper in a peer-reviewed journal? . (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

R eview - Summary:

Discussion of findings:

Since the objective of the investigator was to determine the effect of SO_2 on rheologic parameters, it was appropriate to examine one dosage of SO_2 compared with a control group. However, no explanation was given for the use of 1ppm. Interestingly, hemorheologic changes observed by SO_2 inhalation do not seem to be detrimental to tissue perfusion. Future studies should investigate a gradient of SO_2 exposures and clinical effects on haematological parameters and well as the effect of SO_2 on microrheologic properties of red cells.

Confidence index:

Some aspects of the materials and methodology for this study are not explicit in the paper. Living conditions of the rats prior to the experiment were not mentioned, nor was the process of randomly selecting rats for either the test or control group. No mention was made of an acclimation period prior to testing. In addition, excluded information regarding the age and pre-test health, randomization,blinding etc. may have affected the results in this study. This study was suitable to show whether SO_2 exposure induces acute hematologic and hemorheologic effects at 0.87ppm. Justification for this concentration was not given.

Though there are issues with the study design, this study is valuable in that it provides preliminary evidence as to the effects on blood rheology of SO_2 exposure and information regarding the biological mechanism of SO_2 exposure. The author provides alternate explanations for effects that were observed in this study in addition to SO_2 exposures. Many relevant studies are cited that either agree with or contradict the results of this study. The author also states when comparable studies are not available in the literature. Reasonable assumptions and conclusions are drawn from the evidence. The author gives suggestions for further research. The author does not attempt to draw any major conclusions from these experiments.

Review – Confidence index ranking:

Moderate to high

Author: Gause EM, Barker M	**Internal ID: 193**
Title: Interaction of inhaled sulfur dioxide with mucus glycoproteins	
Year:1978	
Abstract: n/a	
Objective: "We have looked here at uptake of SO_2 by nasal mucus and the effects of SO_2 upon the electrophoretic properties of the nasal mucus glycoproteins subsequent to in vivo inhalation exposure of rats."	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
5-20ppm	**24h/day for 7 days**	**Rat**	**Sprague-Dawley**	**Adult 375-450g**	**Male**	**n/a**	**n/a**	**n/a**	**5ppm**

Observations:

"…data indicate that within the first 30min of exposure, approximately 90% of the inhaled SO_2 remains in the nasal mucus with approximately 10% found in the plasma or serum. At times of 1-4hr after inhalation the ratio of SO_2 concentrations in mucus to that in serum appears to level off at about 3:1." Furthermore, electrophoretic gels of nasal mucus collected immediately following exposure to 5ppm SO_2 show 2 new bands, mucus from rats exposed to 20ppm show 5 new bands. Following 8 days of recovery post-exposure, gels of nasal mucus run following exposure to 5ppm show 3 or more new bands on electrophoresis, and exposures to 20ppm or more show 3-5 new bands. "In this work the appearance of new electrophoretic bands in the acid glycoprotein fraction indicate that inhaled SO_2 has caused the polymerization of mucus glycoprotein molecules resulting in the formation of higher molecular weight aggregates. These molecular aggregates appear to increase in both number and molecular weight or size with increasing concentrations of SO_2."
*Statistically significant

Author's Conclusions:

"In summary, formation of large aggregates of cross-linked glycoprotein molecules by inhaled SO_2 could explain both the decrease in nasal mucus flow rate which has been observed upon SO_2 inhalation in humans, and the increased susceptibility to infection related epidemiologically to SO_2 exposure."

Review – Assessment:

A. Test animals:	-✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? + a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.*

	+b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? -✎ Was the duration of exposure precisely defined? +✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined.

	□ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* ±✏ Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✏ Was the acclimation period specified? Was it of acceptable duration? □ *Guideline: 5 days* -✏ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? -✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? -✏ Was the period of observation following exposure appropriate? □ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? -✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? -✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✏ Was individual data reported for each test animal?
G. Data analysis:	-✏ Are the statistical methods used appropriate? -✏ Are confidence intervals reported? ±✏ Did the investigator appropriately interpret the statistics? ±✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective? +✏ Were assumptions reasonable? +✏ Are the methods reproducible? -✏ Did either the study design or the statistical analysis limit confounding variables? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Study looks at the effect of SO_2 inhalation on the composition of nasal mucus glycoproteins. The formation of large aggregates of crosslinked glycoprotein molecules by inhaled SO_2 could explain both the decrease in nasal mucus flow and the increased susceptibility to infection related epidemiologically to SO_2 exposure. A decrease in nasal mucus flow rate would be expected if the new aggregates lose the cohesive mucus gel structure required to trap and transport invading microbes, leaving the host susceptible to infection. As such, a potential mechanism explaining some of the respiratory effects of SO_2 exposure is proposed. The relevance to humans is that these higher molecular weight aggregates would inhibit mucus flow in the nasal passages leading to an increased susceptibility to infection.

Confidence index:

Exposure assessment was not well defined – no mention of how glycoprotein were isolated from exposed animals. Appears as though only a single animal is used for each experiment. No attempt made to identify glycoproteins, conclusions based on subjective analysis of poorly resolved gels. Gel conditions not defined at all, but again conclusions drawn that new bands represent aggregates of glycoproteins – this is then extrapolated to explain the decrease in nasal mucus flow rate observed upon SO_2 inhalation in humans.

Review – Confidence index ranking:

Low

Author: Citterio, G., Mortola, J. P., Agostoni, E. **Internal ID:** 194

Title: Reflex effects on breathing of laryngeal denervation, negative pressure and SO_2 in upper airways

Year: 1985

Abstract: Respiratory reflex effects of laryngeal denervation, negative pressure and SO_2 in upper airways were studied in anesthetized rabbits. Inspiratory efforts with nasal occlusion had longer duration (TIo) and smaller diaphragm activity (Adi) than with tracheal occlusion. After section of superior laryngeal nerves (SLN) these differences disappeared: values with tracheal occlusion became similar to those with nasal occlusion before denervation. This suggests that laryngeal pressure receptors, firing at zero pressure and decreasing their discharge with negative pressures, increase central inspiratory activity. After SO_2 TIo, both with tracheal and nasal occlusion, increased even after laryngeal denervation, provided SO_2 flowed through nasal pathway. Hence, nose and/or rhinopharynx contain receptors affected by SO_2. After laryngeal denervation and SO2 TIo was shorter with nasal than with tracheal occlusion, despite equal Adi. This, combined with the above findings, suggests two groups of pressure receptors in nose and/or rhinopharynx with opposite effects on inspiratory off-switch: one unaffected and the other probably blocked by SO_2. During nose breathing section of SLN produced only a slight decrease in mean inspiratory flow.

Objective: The first aim of this research was to examine the reflex effects of negative pressure in the upper airways by comparing the inspiratory efforts with tracheal occlusion to those with nasal occlusion in anesthetized rabbits. The next aim was to determine more systematically the reflex effects occurring after SO_2 exposure of upper airways, particularly during inspiratory efforts, in which some differences are more easily detectable. The third aim was to assess whether the reflex effects occurring after SO_2 exposure of upper airways stemmed from laryngeal receptors and/or from supralaryngeal ones.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
300-350 ppm	unknown	rabbits	unknown	unknown	unknown	54	unknown	unknown	unknown

Observations:

After exposure of the upper airways to SO_2, inspiratory time and expiratory time with tracheal occlusion increased*, and the rising rate of diaphragm activity decreased*. These effects lasted for about 30 min after the removal of SO_2. When only the larynx was exposed to SO_2 the increase in inspiratory time was similar to that obtained by laryngeal denervation or by nasal occlusion relative to tracheal occlusion before denervation. At the beginning of exposure to SO_2 of the upper airways 11 out of 15 rabbits coughed for about 10 sec. Nine out of 24 rabbits stopped breathing within about 1 min after exposure, in most of them the apnea did not subside spontaneously within about 10 sec, and ventilation resumed only after light pressure on the rib cage. During apnea the heart rate was 24.2% lower* than at control. At the end of exposure the heart rate of all rabbits was 13.6%* lower than control.

*Statistically significant

Author's Conclusions:

"After SO_2 inspiratory time, both with tracheal and nasal occlusion, increased even after laryngeal denervation, provided SO_2 flowed through nasal pathway. Hence, nose and/or rhinopharynx contain receptors affected by SO_2. After laryngeal denervation and SO_2 inspiratory time was shorter with nasal than with tracheal occlusion, despite equal diaphragm activity. This, combined with the above findings, suggests two groups of pressure receptors in nose and/or rhinopharynx with opposite effects on inspiratory off-switch: one unaffected and the other probably blocked by SO_2. The cough elicited by exposure of the upper airways to SO_2 originates mainly from laryngeal receptors with afferent fibers in the superior laryngeal nerves. SO_2 in the nose or rhinopharynx elicits a defense reflex, probably through stimulation or irritant receptors."

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+/-) c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source unknown *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (-) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and

	housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+/-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+/-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+/-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (+/-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams?

	(+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings indicate that a large part of the reflex effects produced by SO_2 exposure of upper airways stems from supralaryngeal receptors. The location of these supralaryngeal receptors was determined to be in the nose and/or rhinopharynx. Two groups of pressure receptors in the nose and/or rhinopharynx with opposite effects on inspiratory activity: one unaffected by SO_2, the other one probably blocked by SO_2. This study extends the current knowledge of the mechanism of action for SO_2. However, it is not clear what the linkage is between the physiology/anatomy of the rabbit to human responses, nor is that made clear in the paper.

Confidence index:

Observations are generally in agreement with other published works, however, study design was not well defined (precise dose and duration of exposure not known). Therefore, the experiment would not be easily reproducible. No attempt to limit confounding variables: sex, age, health and weight were not controlled. The results were presented in a very verbose and unstructured manner. In addition, The rabbits were not randomly assigned to exposure groups and there was no information on the control regime. In Series 5 experiments, 5 rabbits were chosen from Series 4, but there is no information on how these rabbits were chosen. In Series 4, attempts were made either to bypass the rhinopharynx-nose pathway or to fully convey the SO_2 mixture through it. Only 3 rabbits were used, but there is no information on how the rabbits were chosen. There is practically no information on the exposure conditions, including the exposure duration. Some of the rabbits were handled differently. For example, when it was discovered that 2 rabbits were mouth breathing, there mouths were manually occluded to force nose breathing.

Review – Confidence index ranking:

Low to moderate

Author: Citterio G. Piccoli S. Agostoni E	**Internal ID: 195**

Title: Breathing pattern and diaphragm EMG after SO_2 in rabbit intra- or extrathoracic airways.

Year: 1985

Abstract: In anesthetized rabbits under SO_2 block of slowly adapting stretch receptors (SAR) in intrathoracic airways TI increased by 49.7 +/- 6.9% and VT by 19.5 +/- 3.7%. TE (expiratory time) decreased for small increases of TI (inspiratory time) and vice versa. Slope of moving average of diaphragm EMG (M.A.) decreased by 16.7 +/- 4.1% from 0 to 30% TI control, and increased by 26.3 +/- 8.1% from 30 to 70%. Considering that discharge from intrathoracic SAR increases during inspiration, these changes of M.A. indicate that their input facilitates inspiration at its beginning, but inhibits it later on, in line with previous conclusions based on SAR block which included 2/3 of extrathoracic trachea (ETT). Neither SO_2 nor pressures from -40 to +40 cm H_2O in ETT changed breathing pattern or M.A.. Hence, SAR of ETT do not affect parameters studied in rabbits. When extrathoracic airways were exposed to SO_2 TI increased by 30.5 +/- 8.2%, TE by 37.9 +/- 5.1%, VT did not change. Hence, some receptors rostral to trachea, involved in respiratory control, are affected by SO_2.

Objective: "…we compared the effect on the breathing pattern and the electrical activity of the diaphragm produced by exposure to SO_2 of either the thoracic airways or the extrathoracic trachea… A second aim of the present research was to check whether the exposure to SO_2 of the airways cranial to the trachea has any effect on the breathing pattern and the electrical activity of the diaphragm."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
300-350ppm	**15-20min**	**Rabbit**	**White**	**n/a 1.5-2.5kg**	**n/a**	**28**	**healthy**	**n/a**	**n/a**

Observations:

SO_2 was used in this study to block slowly adapting stretch receptors in rabbits, rather than being the focal point of the study itself.
Thoracic Airways:
"The increase of Ti 49% and VT (tidal volume) (19%), the lack of significant decrease of TE (expiratory time) produced by SO_2 block confirm the findings previously obtained…"
"TE decreased when the increase of TI (inspiratory time) produced by the SO_2 block was small, while it increased when the increase of TI was large."
Extrathoracic Airways:
"No significant changes of TI, TE and VT occurred after exposure of the extrathoracic trachea (ETT) to the SO_2 mixture…"
The remainder of results are too complex to present here. Please see Abstract for a summary.

*Statistically significant

Author's Conclusions:

"There is no evidence in the upper airways of rabbits of slowly adapting stretch receptors similar to those of the tracheobronchial tree. Hence, one cannot know whether the changes occurred after exposure of ETAW (extrathoracic airways) to SO_2 were due to receptors block or other mechanisms, and where SO_2 acted…

[Based on the findings of another experiment] One could, therefore, argue that that the effects produced by SO_2 in our experiments should originate cranially to the larynx. On the other hand, the concentration of SO_2 used by these authors is 1/3 of ours and the time of exposure is not given. It seems, therefore, possible that the effects of SO_2 in our experiments could originate from the larynx."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? -✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? ±✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%*

	-✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* ±✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics?

	+✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? -✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Determined that the slow adapting stretch receptors of extrathoracic trachea are also blocked by SO_2, similar to airways slow adapting stretch receptors. Also demonstrated that exposure to SO_2 of the airways cranial to the trachea caused an increase in both inspiratory and expiratory time, as well as, decrease in the moving average of the diaphragm EMG. However, the authors were unable to localize exactly where the effect occurred, though they hypothesized it occurred in the trachea or at a location superior to the trachea. While demonstrating some possible effects of SO_2 exposure in rabbits, the findings are not conclusive enough to provide any definitive and specific conclusions.

Confidence index:

This paper is very long and poorly translated, making it difficult to read. Results are presented in a convoluted and confusing manner. While the experiments themselves seem to have been conducted in a rigorous manner, the way the paper is written makes it very difficult to determine the overall quality of the study findings. Many of the acronyms used in the study were not defined, making it very difficult for someone not in the field to interpret the data correctly. The report was also very poorly written, also verbose and repetitive. No attempt to limit confounding variables, unknown sex, age, weight and no mention of random assignment of controls and exposed.

Review – Confidence index ranking:

Low to Moderate

Author: Barthelemy P. Badier M. Jammes Y.

Internal ID: 197

Title: Interaction between SO_2 and cold-induced bronchospasm in anesthetized rabbits.

Year: 1988

Abstract: In anesthetized, paralyzed and artificially ventilated rabbits, reflex changes in lung resistance induced by cooling the inspired air from 38 to 15 degrees C were studied before and after 45 min periods of SO_2 exposure at two different concentrations (0.5 or 5 ppm). Both concentrations of SO_2 induced significant increase in RL in intact animals (+16% and +50%, respectively). The effect of 5 ppm SO_2 persisted after vagotomy. The cold-induced bronchospasm was halved after exposure to 0.5 ppm SO_2 and was no longer significant after exposure to 5 ppm SO_2. In both cases, RL recovered to control values 40 min after the end of SO_2 exposure and then, the magnitude of cold-induced bronchospasm also recovered. The reflex bronchoconstrictor response to phenyldiguanide (PDG) i.v. disappeared after exposure to 5 ppm SO_2. However, the bronchomotor response to histamine i.v. which involved both reflex and direct actions on airway smooth muscle, was not altered. These results show that (1) prolonged increase in RL measured after SO_2 exposure does not result from a vagal reflex; (2) the cold-induced bronchospasm, as well as the bronchomotor response to PDG, are reduced or suppressed during the period where the effect of SO_2 persisted. This suggests that 45 min exposure to SO_2 induces transient alterations in tracheobronchial wall, which reduce the accessibility to nervous receptors in the airways.

Objective: "The aim of the present work was to study on an animal model the changes in cold-induced bronchospasm following moderately prolonged inhalation of SO_2 at two concentrations (0.5 and 5.0ppm), corresponding to those measured during moderate or intense urban traffic. This interaction was also studied after vagotomy in order to suppress vagal reflexes which may be inactivated by SO2 inhalation."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0.5-5.0ppm	**45min @ 0.5ppm** **45min @ 5.0ppm**	**Rabbit**	**n/a**	**Adult** **2.0±0.2kg**	**n/a**	**19**	**n/a**	**n/a**	**0.5ppm**

Observations:

"Following inhalation of 0.5ppm SO_2 for 45min, RL (lung resistance) values increased from 21.0 ± 1.6(SE) to 24.6±0.8(SE) cmH20/l s (+16%, $P<0.05$). Inhalation of 5ppm SO_2 produced a more important increase in RL, which rose from 2.09±2.0SE to 30.6±3.4SE cm H20/l s (+50%, $P<0.01$). These SO_2-induced changes in RL were measured for 20min after SO_2 inhalation ceased. Then RL returned progressively to control values for both concentrations about 40min after the end of each exposure."

0.5ppm SO_2 exposure reduced cold-induced bronchospasm by 23% ($p<0.01$) and 5.0ppm SO_2 reduced it by 43% ($p<0.001$).

5.0ppm SO_2 exposure did not significantly modify the bronchomotor effect produced by the two doses of histamine.

5.0ppm SO_2 exposure induced the same increase in RL after cervical bivagotomy as before.

"The present results show that inhalation of a dry gas mixture containing 0.5 or 5ppm SO_2 for a 45min period produced a gradual increase in lung resistance but halved (with 0.5ppm) or suppressed (with 5.0ppm) the cold-induced bronchoconstriction. This study also shows that SO_2 exposure (5ppm) abolishes the bronchomotor response to PDG iv. But does not modify the response to histamine iv. The effects of SO_2 on lung resistance and bronchomotor responses to cold air or PDG are observed only for the 40min period which follows SO_2 exposure."

*Statistically significant

Author's Conclusions:

"In conclusion, the present results suggest that the exposure to an atmospheric pollutant does not enhance but reduces the bronchomotor response to chemicals or cold air breathing. This protective effect may result from a non-specific action of SO_2 on the tracheobronchial wall."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* +b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
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C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers

	appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✏ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✏ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
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E. Procedural:	±✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* ±✏ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? -✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
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G. Data analysis:	+✏ Are the statistical methods used appropriate?

	±✓ Are confidence intervals reported? +✓ Did the investigator appropriately interpret the statistics? +✓ Are the observed trends statistically significant?
H. Interpretations:	+✓ Was the original objective addressed? -✓ Are the results novel? +✓ Was the design of the study appropriate to investigate the primary objective? +✓ Were assumptions reasonable? +✓ Are the methods reproducible? +✓ Did either the study design or the statistical analysis limit confounding variables? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

A population (n=19) of rabbits was exposed to extreme values of air temperature (8-38 C) and to 0.5 and 5 ppm SO_2 in order to define the relationship between SO_2 and air temperature, specifically cold-induced bronchospasm. The study results support the findings of other studies that SO_2 chronic exposure suppressed cold-induced bronchospasms. The paper goes on to suggest that inflammation of the respiratory epithelium may be responsible for a decreased accessibility of inhaled substances. This study provides a good weight-of-evidence argument that chronic exposure to SO_2 can decrease airway responsiveness to mediators or physical agents to sensory nervous endings present in the epithelium and airway mucosa. SO_2 seems to reduce the bronchomotor response to chemicals or cold air breathing, by acting on the tracheobronchial wall.

Confidence index:

While animal characteristics are not as complete as guidelines request, the methods contained within this paper appear to be of good quality, with sufficient sample size (n=19), good control groups, and rigorous statistical analyses. The results contradict several human studies, which report a potentiation of changes in lung mechanics by the inhalation of cold air after exposure to SO_2. Although the previous studies used short duration of exposure to SO_2 compared to this study. Still the authors should have performed a similar expt before concluding that SO_2 has a protective effect on the bronchomotor response to cold air breathing. Nevertheless, the exposure methods and assessment were well defined – should be easily reproducible. Attempt to limit confounding variables by using each rabbit as its own internal control. Use of relevant doses of SO_2, similar to those found in intense urban traffic. Used a single endpoint – lung resistance as a measure of bronchomotor response.

Review – Confidence index ranking:

High to moderate

Author: Asmundsson, T., Kilburn, K.H., McKenzie, W.N.	**Internal ID:** 198
Title: Injury and Metaplasia of Airway Cells Due to SO_2	
Year: 1973	

Abstract: Abrupt exposure of Sprague-Dawley rats and Syrian hamsters to 400 ppm of sulfur dioxide (SO_2) caused death within 6 hours in 22 of 30 rats and six of six hamsters. It was difficult to assign the damage, which was observed in survivor rats to SO_2 because patchy pneumonia was seen and pathogenic bacteria were grown from many tracheas or lungs. Because of these problems hamsters were used and bacterial cultures of lung and trachea were usually sterile. Sequences of epithelial changes were seen in the trachea and large bronchi with exposure to 100, 200, or 400 ppm for up to 6 weeks. For example, at 200 ppm there was: 1. Loss of cilia, vacuolation, pyknosis, and extrusion of cells after 1 to 2 days, 2. Only transient goblet cell hyperplasia after 3 to 4 days, 3. Basal cell hyperplasia and the development of stratified squamous epithelium in 6 to 8 days, and 4. Squamous metaplasia after 2 to 4 weeks. Higher doses (400ppm) produced squamous metaplasia after 1 to 2 weeks. Goblet cell metaplasia appeared to be a brief phase in the response to damage which produces squamous metaplasia. Exposure to 40 ppm SO_2 alone for 4 hours per day for 6 weeks produced no changes, but 24 hours after a 4-hour exposure to 40ppm SO_2 plus 0.74 gm of carbon dust per cu. M. of air, neutrophils were recruited into airway epithelium. It is suggested that this synergistic effect is due to the carbon particles adsorbing SO_2, which leaches off slowly, perhaps within macrophages, to recruit leukocytes in airways.

Objective: This study was done to answer the following questions: (1) What morphologic changes are produced by repeated injury to airway epithelial cells by SO_2 alone? (2) What is the time course of these morphologic features? (3) Do they resemble goblet cell hyperplasia and squamous metaplasia? (4) Can simultaneous exposure to carbon particles enhance the injurious effects of SO_2? (5) Can a model of acute bronchitis be created which is not due to infection? (6) Are acute bronchitis and chronic bronchitis related in this model?

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
400 ppm SO_2	5hr/d,5d/wk,23d	Rats	Sprague-Dawley	Young	Male	30	No info	N/A	N/A
Filtered room air						6			
400 ppm SO_2	5hr/d,5d/wk	Hamsters	Syrian	6-8 wks	No info				
40 ppm SO_2	5hr/d,5d/wk/6wk					6			
100 ppm SO_2	5hr/d,5d/wk/6wk					6			
200 ppm SO_2	5hr/d,5d/wk/6wk					6			
250,330,350 and	5hr/d,5d/wk,7wk					6			
400 ppm SO_2						7			
40 ppm SO_2	5hr/d,5d/wk,8d								
200 ppm SO_2	5hr/d,5d/wk/8d					8			
40 ppm SO_2	4 hr					4			
40 ppm SO_2 +						8			

carbon dust	4 hr								
Carbon Dust	4 hr					12			
Filtered room air						8			
						43			

Observations:

Rats exposed to 400 ppm SO_2

Three rats died at the end of the first 5hour exposure and a total of 22 rats died spontaneously during the 1st week. The lungs and trachea appeared normal in 11 of the 13 rats studied. Some of the changes in the trachea included epithelial degeneration, loss of cilia, infiltration of leukocytes, hemorrhage, and extensive cellular necrosis and desquamation, extensive squamous metaplasia.

Only 2 of the 6 control rats had normal tracheas. Because of the problems with the control rats, the experiments were continued in the hamsters only.

Hamsters exposed to 400 ppm SO_2

The six hamsters exposed acutely to 400 ppm SO_2 immediately developed respiratory distress. The six hamsters were all dead after 6 1/3 hours of exposure. Pathological changes were similar to those described in the rats.

Experiments to Determine Tolerance to SO_2 and Airway Changes in Hamsters During Long-Term Exposure

Because abrupt exposure of hamsters to 400ppm SO_2 caused 100% mortality during the first or second 5hr exposure, the dose of SO_2 was gradually increased from 250 to 400 ppm over a period of 4 days.

The exposure to 40,100,200,and 400 ppm SO_2 produced epithelial damage in large airways in all groups after 1 week of exposure.

A single 5-hour exposure to 200 ppm of SO_2 produced disappearance of almost all cilia in the trachea.

Four days of exposure for 5 hours per day to 200ppm resulted in a significant reduction in the number of goblet cells in the trachea.

After 1 week of exposure to SO_2 with a gradual increase in dose from 250 to 400 ppm many ciliated cells were being extruded and 18 percent of tracheal cells were goblet cells. After 3 and 4 weeks, 20.3% and 17% of tracheal cells were goblet cells, respectively, but neither showed a significant difference from controls ($p<1.0$). After 3 to 6 weeks of exposure goblet cells were decreased significantly only in tracheas of hamsters exposed to 200 ppm.

Studies of time course of Changes due to 40 and 200 ppm SO_2

Ciliary loss was seen after 1 day of expose to both 40 and 200 ppmSO_2. These doses of SO_2 did not produce the leukocyte recruitment into the trachea which occurred after a few hours at 400ppm.

Acute airway changes after 40ppm SO_2 alone and with carbon dust.

Four-hour exposure to SO_2 alone produced no leukocyte recruitment. After 44 exposure hours, the changes still were not significantly different from the controls. The combination exposure results in numerous polymorphonuclear leukocytes within bronchial walls and in the lumen 24 hours after beginning exposure.

*Statistically significant

Author's Conclusions:

In hamsters, continued daily exposure for 200 or 400 ppm of SO_2 changed epithelium to squamous but did not make goblet cells persist.

It is suggested that the synergistic effect between SO_2 and carbon dust is due to the carbon particles adsorbing SO_2, which leaches off slowly, perhaps within macrophages, to recruit leukocytes in airways.

Review – Assessment:

A. Test animals:	(-)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation *Guideline: Below 20% variation* (-) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective?

	(+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (+) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (+)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water May be withheld optionally.*
D. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(+/-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (+)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups?

	(+/-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random Assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+/-)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+/-) Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+/-)Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (+) Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially

	bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

In this study, the sequence of airway epithelia changes after exposure to nonfatal doses of sulfur dioxide was first dilation and vacuolation and extrusion exfoliation of ciliated and goblet cells in a few hours. The final change was squamous metaplasia of epithelium after 2 to 4 weeks of exposure to SO_2 at levels above 200 ppm. Higher doses of SO_2 produced squamous metaplasia after 1 to 2 weeks. Exposure to SO_2 and carbon dust resulted in neutrophils being recruited into the airway epithelium. The doses used in this study are higher than concentrations typically encountered by humans. There is minimal raw data included in the study, so the magnitudes of the changes are not known. Few of the results are statistically significant. Also, the hamster is a poor model because it is purported to have undeveloped tracheal and bronchial mucous glands – something the authors themselves point out, hamster is also extremely resistant to SO_2. However, this paper could serve as a benchmark for what happens in one animal species (hamsters) at increasing levels of SO_2.

Confidence index:

The sample sizes used in this study are small. Furthermore, the sex of the hamsters is not given and the weight variation of the hamsters is greater than 20%. There is interindividual variability in the responses and the animals are not randomly assigned to exposure groups. It is not specified if different animals are used for each exposure group and there is minimal raw data included in the paper.

Despite not accounting for a number of confounding factors, the weight changes of the animals were monitored throughout the study. In addition, the author provides evidence to justify the use of hamsters for this study and does not extrapolate to humans.

Review – Confidence index ranking:

Low

Author: Okuyama H. Majima Y. Dannenberg AM Jr. Suga M. Bang BG. Bang FB.	**Internal ID: 199**

Title: Quantitative histological changes produced in the tracheal mucosa of young chickens by the inhalation of sulfur dioxide in low concentrations.

Year: 1979

Abstract: Chickens were exposed to SO_2 in relatively low concentrations (3.4 to 18.5 parts per million (ppm)) for 1 to 14 days. A portion of their tracheas was embedded in water-soluble methacrylate, cut at 2 micrometer and stained with hematoxylin and eosin, Wright's stain, methyl green-pyronin, Alcian blue - periodic and Schiff, and for acid phosphatase. An increase was found in (a) the mucosa to wall ratio; (b) the number of mucosal cells in mitosis; (c) the number of macrophages, lymphocytes, plasma cells, and neutrophils in the epithelium and lamina propria; and (d) the number of these infiltrating cells which contained acid phosphatase. The number of mucus- and seromucus- secreting cells and vasoamine-containing cells were sometimes increased, but not consistently. The percentage of cells containing sialidase-sensitive sialomucins was elevated, and percentage of cells containing neutral mucins was reduced. These changes were only partly related to the SO_2 concentration and the duration of SO2 exposure, in that increasing amounts of SO_2 did not always cause increasing changes in the mucin composition. Evidently, the altered mucins sometimes protected against further mucin modification.

Objective: "The present report describes the effect of various, relatively low concentrations (3.4-18.5ppm) of SO_2 on the tracheal mucosa of young chickens."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
3.4-18.5ppm	**1-14 days (16-22h/d)**	**Chicken**	**White leghorn**	**2-4 weeks**	**n/a**	**n/a**	**n/a**	**n/a**	**3.4ppm**

Observations:

Cells in Tracheal Mucus Membranes
- mucosa to wall ratio doubled at highest concentration (18.5ppm) for 14 days and was not changed the lowest exposure of 3.4ppm for 22hrs
- infiltrating mononuclear and polymorphonuclear cells increased at all exposure levels
- number of acid phosphatase positive cells increased at all exposure levels
- number of plasma cells and lymphocytes increased with SO_2 exposure
- number of mitotic figures was increased significantly with SO_2 exposure

Changes in mucus type of tracheal mucus membranes
- neutral mucins decreased while acidic mucins increased

With exposures of 18.5ppm for 7 days, some chickens showed mucosal damage

*Statistically significant

Author's Conclusions:

"In our experiments with young chickens, as with rats, SO_2 inhalation produced a slight increase in the number of mucus and sero-mucus secreting cells in the surface epithelium and glands, accompanied by an increase in their mitotic index."

"The lack of a consistent dose-response curve deserves comment. It probably results from a compensatory response on the part of the exposed host. Changes in the amount and type of mucus may give added protection to the mucosa and thickening of the mucosa may give added protection to the underlying tissues. The increased protection would lessen the stimulus that SO_2 gives the cells, causing less mucus to be produced. Thus, a fluctuating response to SO_2 occurs with time, and this has been consistently observed in chicken nasal mucus membranes."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	±✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? -✎ Was the duration of exposure precisely defined? +✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? ±✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated?

	☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* +✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* +✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* -✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? +✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? +✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? ±✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?

H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? -✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

An undetermined number of chicks (1 day old chickens) were exposed to varying concentrations of SO_2. Results did not differ significantly from that found in rats. Results show that inhalation of SO_2 produces a slight increase in the number of mucus and seromucos secreting cells in the surface epithelium and glands, accompanied by an increase in their mitotic index. The number of ciliated cells appeared relatively decreased. These may be characteristics of chronic bronchitis in general. The increase in sialidase-sensitive sialomucins with SO2 is in contrast to findings with the effect of tobacco smoke in human and rat – suggest chicken may not be a good model to extrapolate from. While clear dose response relationships did not exist for all response variables measured, this can likely be attributed to individual variation. Still, the authors have identified potential responses in an animal model following SO_2 exposures, and have further identified methods by which tissues attempt to compensate for SO_2 induced damage.

Confidence index:

Exposure methods and assessment are not well defined – may not be reproducible. The methods of exposure, sample size, and animal model characteristics are described rather poorly, while the methods of measuring effect are described in great detail. Using histological and histochemical methods for assessment makes it very subjective. Lack of consistent dose-response curve and exposure time curve is puzzling. Authors themselves state species specific differences in response to SO_2 – chicken is not a good model to extrapolate from.

Review – Confidence index ranking:

Low to Moderate

Author: Matsumoto, S., Takeda, M., Saiki, C., Takahashi, T., Ojima, K.	**Internal ID:**200

Title: Effects of Vagal and Carotid Chemoreceptor Afferents on the Frequency and Pattern of Spontaneous Augmented Breaths in Rabbits

Year: 1997

Abstract: We investigated the effects of vagal afferents and carotid chemoreceptors on the frequency and pattern of spontaneous augmented breaths in anesthetized rabbits by analyzing the changes in the phrenic nerve discharge. Blockade of vagal C-fiber conduction by capsaicin treatment greatly reduced the frequency of spontaneous augmented breaths but had no significant effect on the pattern of the augmented breaths. Inhalation of sulfur dioxide, which abolished the Hering-Breuer inflation reflex, suppressed the frequency of spontaneous augmented breaths but did not significantly alter the pattern of the augmented breaths. Carotid sinus denervation diminished both the frequency and amplitude of spontaneous augmented breaths. The occurrence of spontaneous augmented breaths was not observed in rabbits after bilateral vagotomy. In other experiments we examined the relationship between rapidly adapting pulmonary stretch receptors (RARs) and dynamic lung compliance during the spontaneous augmented breath. The stimulation of RARs occurred at the first phase of spontaneous augmented breaths, and augmentation of transpulmonary pressure seen at the second phase coincided with vigorous stimulation of the receptor activity. These results suggest that spontaneous augmented breaths may be mediated by the summation of several facilitatory inputs from vagal and carotid chemoreceptor afferents.

Objective: To evaluate the influences of vagal and carotid chemoreceptor afferent inputs on the frequency and pattern of spontaneous augmented breaths in anesthetized rabbits. The frequencies of spontaneous augmented breaths and the changes in the phrenic nerve activity seen at the augmented breath were examined before and after perineural application of capsaicin to the vagus nerves. Furthermore, the changes in the same respiratory parameters were compared before and after inhalation of SO_2, surgical carotid sinus denervation, or bilateral vagotomy. Finally, the effects of spontaneous augmented breaths on the relationship between rapidly adapting stretch receptor activity and dynamic lung compliance were examined in animals with unilateral vagotomy.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
200 ppm	unknown	Rabbit	unknown	unknown	Male and female	6	unknown	No observed adverse effect level	Lowest observed adverse effect level

Observations:

Before inhalation of SO_2, lung inflation with 2 V_T (tidal volumes) abolished PNA (phrenic nerve activity), but this respiratory effect disappeared after SO_2 treatment.

SO_2 inhalation abolished the Hering-Breuer reflex and suppressed the frequency of spontaneous breaths.

SO_2 inhalation did not significantly alter the pattern of augmented breathes.

*Statistically significant

Author's Conclusions:

The origin of a spontaneous augmented breath may be related to the afferent information from SARs (stretch receptors). It is also reasonable to expect that an increase of V_T seen after blockade of SARs due to SO_2 inhalation may be equivalent to a "mini" augmented breath with each inspiration., Therefore, the increased V_T would improve compliance and lead to a less frequent occurrence of spontaneous augmented breaths.

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+/-) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex: (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (-) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+/-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+/-) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+/-) Was the duration of exposure appropriate to investigate the primary objective? (+/-) Was the duration of exposure precisely defined? (N/A) If exposure chambers were employed, were animals exposed during the

	equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+/-) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(N/A) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (N/A) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (N/A) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+/-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+/-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(N/A) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? (N/A) Were test animals randomly assigned to exposure groups? Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+/-) Was a control group assayed?

	(+/-) Is the control regime appropriate? (+/-) Was the period of observation following exposure appropriate? - *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+/-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+/-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study attempted to identify afferent mechanisms that provoke spontaneous augmented breaths. Inhalation of sulfur dioxide, which abolished the Hering-

Breuer reflex, suppressed the frequency of spontaneous augmented breaths but did not alter their pattern. Data suggests that the origin of spontaneous augmented breaths may be related to afferent information from slowly adapting stretch receptors. The suppression of the Hering-Breuer reflex was initiated in six rabbits by the inhalation of 200 ppm SO_2. This benchmark was then used to time the frequency, pattern and timing of the spontaneous augmented breath reflex that is used by the lung to reopen collapsed alveoli. The study found that the exposure to 200 ppm SO_2 did not significantly affect the timing or pattern, but did significantly affect the frequency. The applicability of this study to human exposure seems tenuous, as there is no justification provided on why rabbits were used as study animals, and other problems related to the conduct of the study.

Confidence index:

This was a novel study designed to identify mechanisms that maintain spontaneous augmented breath. Lack of history on the test animals before experiment commencement, lack of source of animals and reagents, lack of justification on the use of rabbits over rats, lack of gradation of SO_2 exposure detract from the confidence in this study. Used levels of SO_2 known to block slowly adapting stretch receptors. The researchers attempted to limit confounding variables by having each rabbit act as its own internal control. Exposure conditions and assessment methods were well defined and well established (with the exception of duration of exposure). Consequently, the study should be easily reproducible.

Review – Confidence index ranking:

Moderate

Author: Gause EM, Rowlands JR	**Internal ID: 201**
Title: Effects of sulfur dioxide and bisulfite ion upon human lymphocyte membranes.	
Year: 1975	
Abstract: n/a	
Objective: "We have been concerned with the molecular level effects and mechanisms of effects of sulfur dioxide upon lymphocyte membranes, and present here the results of a spin label study of this system."	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
1.34×10^{-5} to 1.34×10^{2} mol	**n/a**	**Cloned human lymphocytes**		**n/a**	**n/a**	**n/a**	**n/a**		

Observations:

When lymphocytes labeled with the spin label 4-isothiocyanato-2,2,6,6-tetramethyl-piperidinooxyl were resuspended in increasing concentrations of SO_2, a dose-dependent spectral change was observed. The effect did not appear to be reversible by washing the cells with fresh media. SO_2 also increased the aggregation of membrane surface protein as extrapolated from the marked increase in exchange broadening induced by SO_2. No evidence for cross linking of membrane proteins could account for the observed SO_2 effects.
*Statistically significant

Author's Conclusions:

Upon exposure of labeled lymphocytes to SO_2, the spin label spectra reflected a progressive increase in the degree of line broadening leading to the appearance of a broad single line spectral feature. We believe these observations indicate formation of microparticles or microaggregates of membrane protein structures at the lower concentration levels leading to the appearance and growth of the broad single line species as the size and extent of aggregate formation proceeds with increasing SO_2 concentration. Chronic exposure to low levels of SO_2, thus, could induce unnaturally high rats of turnover of lymphocyte membrane proteins, which in turn, would demand increased rates of protein synthesis. The implications of the ability of inhaled SO_2 to significantly and nonspecifically alter lymphocyte membrane protein are extensive.

Review – Assessment:

A. Test animals:	+/-✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? ±a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.*

	±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* ±c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? -✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
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E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* ±✎ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? ±✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? ±✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? ±✎ Was individual data reported for each test animal?
G. Data analysis:	±✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? ±✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This paper discusses the cellular (human lymphocyte) reaction to different species of sulphur compounds (dioxide and bisulfite ion) at varying concentrations. It provides good information on what was determined to be the lymphocyte reaction to sulphur dioxide and to bisulfite suggesting that, because of small differences in cells exposed to varying concentrations of the two compounds that other species of sulfur compounds could also be present. Data supports previously published works that indicate SO_2 can affect the normal immunological functions by damage of peripheral blood lymphocytes. Novel study looking at SO_2 induced protein aggregation by monitoring spectral change of a spin label in human lymphocytes. Data supports the role of inhaled SO_2 in reducing immunological function, likely through loss of immunological specificity (altered recognition).

Confidence index:

Novel study to study the effects of SO_2 on lymphocyte membranes. Should have also looked at additional parameters to support the theory of SO_2 induced protein aggregation. Also should have looked at multiple lymphocyte cell lines to characterize this effect. Exposure to SO_2 was not well defined in terms of duration and how dissolved SO_2 was prepared.

Review – Confidence index ranking:

Moderate

Author: Singh J	**Internal ID: 203**
Title: Teratological evaluation of sulphur dioxide.	
Year: 1982	
Abstract: Sulphur dioxide is a common air pollutant gas. Experiments were carried out to evaluate the teratogenicity of sulphur dioxide in mice. Pregnant animals were exposed to 0, 32, 65, 125, and 250ppm of sulphur dioxide gas from gestation day 7-17. The animals were housed in plexiglass environmental chambers. The environmental chambers were equipped with an airtight see-through door and two gascock openings at diagonal ends for quick flushing with gases. The concentration of the gas was frequently monitored by Beckman Infrared Analyser Model 865. The gas mixture cylinders were obtained from Union Carbide and the cylinders were equipped with two stage regulators and micrometers. The animals were sacrificed on gestation day 18 and fetuses were studied for any teratological effects. A careful examination of the fetuses revealed that exposure to sulphur dioxide gas did not produce any significant effect on the number of dead or resorbed fetuses and the live pups did not show any significant teratological changes. However, a significant number of fetuses had hematomas at all levels of exposure and spinal hematomas at low levels of exposure.	
Objective: Experiments were carried out to evaluate the teratogenicity of sulphur dioxide in mice.	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0-250ppm	**11 days**	**Mice**	**Albino CD-1**	**Adult**	**female**	**74**	**Pregnant**	**250ppm**	**n/a**

Observations:

Exposure of pregnant animals to SO_2 at these concentrations did no produce any apparent signs of maternal toxicity during gestation. A careful examination of the fetuses revealed that exposure to sulphur dioxide gas did not produce any significant effect on the number of dead or resorbed fetuses and the live pups did not show any significant teratological changes. However, a significant number of fetuses had general hematomas at all levels of exposure and spinal hematomas at low levels of exposure. There was a significant decrease in weight of pups exposed to 65 and 125 ppm SO_2*, in contrast, 250 ppm caused a significant
*Statistically significant

Author's Conclusions:

A careful examination of the fetuses revealed that exposure to sulphur dioxide gas did not produce any significant effect on the number of dead or resorbed fetuses and the live pups did not show any significant teratological changes. However, a significant number of fetuses had general hematomas at all level of exposure and spinal hematomas at low levels of exposure.

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study?

	+a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex +d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✏ Were the chosen exposure levels appropriate to investigate the primary objective? -✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? ±✏ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? +✏ Was evidence provided to justify the dosing regime? -✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✏ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✏ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✏ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* +✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* +✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water*

	may be withheld optionally.
D. Equipment:	-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? +/-✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? +✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? ±✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +/-✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? ±✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? -✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +/-✎ Are the extrapolations reasonable?

	+✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

A population of n=74 pregnant mice were exposed to varying concentrations of SO_2 (0, 32, 65, 125, 250ppm) from the 7-17th day of gestation. These exposure concentrations did not seem to create any teratological effects in the unborn young, other than an increase in the frequency of hematomas at the 32, and 65 ppm level. These findings indicate that there is some interaction between levels of SO_2 in the mother and the effect on the fetus, however what that interaction could be is not hypothesized in this paper. The fact that at greatest concentration (250 ppm) there is the largest average weight (1.13 g) and greatest length (2.10 cm) of fetus and the lowest number of hemtomas (3.5%) seems to indicate a beneficial or protective effect of SO_2, although this is not discussed directly in the paper.

Confidence index:

This paper is written up as a short note, and as such the methods, results and discussion are presented in a very brief manner. Novel experiment to study effects of SO_2 exposure on prenatal development in mice. No justification for doses used especially considering introduction focused on levels of ambient SO2. Not too many parameters assessed – live versus dead pups and number of hematomas. Only significant data was changes in weight and length, but no comment made on what the significance of this was. No attempt made to interpret any of the data.

Review – Confidence index ranking:

Moderate to low

Author: Amdur, M., Ugro, V., Underhill, D.	**Internal ID:** 204
Title: Respiratory response of guinea pigs to ozone alone and with sulfur dioxide	
Year: 1978	
Abstract: This study was designed to utilize our guinea pig bioassay method for irritant response to address the question of whether or not ozone and sulfur dioxide appeared to react to form sulfuric acid in the respiratory tract. Animals were exposed to 0.2, 0.4 and 0.8 ppm of each gas alone and to the combination at concentrations of 0.4 and 0.8 ppm. In these experiments sulfur dioxide alone produced no statistically significant alterations in respiration. All concentrations of ozone produced an increase in respiratory frequency. At the two higher concentration the increase in frequency was accompanied by a decrease in compliance. The response to the combinations was the same as the response to those levels of ozone alone. No sulfuric acid was detected in the chamber atmosphere. The biological data suggest that none was formed in the lung.	
Objective: To address the question of whether or not ozone and sulfur dioxide appeared to react to form sulfuric acid in the respiratory tract.	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0.2, 0.4, 0.8 ppm	2 hour	Guinea pig	Random bred	NA	NA	30	NA	All concentrations	No adverse effects were documented

Observations:

In these experiments sulfur dioxide alone produced no statistically significant alterations in respiration. All concentrations of ozone produced an increase in respiratory frequency. At the two higher concentration the increase in frequency was accompanied by a decrease in compliance. The response to the combinations was the same as the response to those levels of ozone alone. No sulfuric acid was detected in the chamber atmosphere. The biological data suggest that none was formed in the lung.
*Statistically significant

Author's Conclusions:

Results do not indicate a synergism between ozone and sulfur dioxide as evaluated by the respiratory response of guinea pigs under our exposure conditions, in which no detectable sulfate or sulfuric acid aerosol was formed in the exposure chamber. The fact that the response of the animals to the combination of ozone and sulfur dioxide could be accounted for on the basis of the response to the ozone component suggests that the two gases do not interact in the respiratory tract of the guinea pig to form sulfuric acid. Our data on guinea pigs do not rule out this (synergism between ozone and sulfur dioxide) possibility in human subjects. Our data do, however, make this explanation unlikely.

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? + *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+/-) c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (-) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+/-) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+/-) Was the frequency of administration appropriate to investigate the primary objective?

	(+/-) Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+/-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+/-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(+/-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (+/-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+/-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+/-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and

	dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+/-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+/-) Was the timing and frequency of assessment logical? (+/-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(N/A) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (N/A) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
H. Interpretations:	(+/-) Was the original objective addressed? (-) Are the results novel? (+/-) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study demonstrated that exposure of guinea pigs to .2, .4 or .8 ppm had no effect on guinea pig lung mechanics. These concentrations are similar to those found to have no effect on human respiration. However, in some measures (specifically in frequency of breaths/min and in Minute Volume) the 0.8 ppm group did exhibit the greatest difference from the 0.2 ppm group (18 breaths/minute and 49 mL difference) does suggest that increased mechanical stress (not biochemical) is placed on the animal. However, no statistical test results were presented on these comparisons. This could have a bearing on human response to SO_2 in that it does indicate that low levels of SO_2 could cause increased breathing response, that could mechanically stress the cardiovascular system, without having an attendant, measurable biochemical response.

Confidence index:
Exposure conditions and assessment methods were simple and well defined – study should be easily reproducible. Additional parameters, longer exposure times, longer post exposure times could have been looked at to more thoroughly assess SO_2 effects on lung function. Each animal served as its own internal control for comparison of exposure effects. It should be noted that the objective of the study was not to look at SO_2 effects alone, but rather its ability to synergize with ozone. The study did test for the objective, however the lack of information on statistical analysis, the fact that a control group was not assayed, instead a ½ hour acclimation period was used as a control regime, the lack of comparison between exposure groups, tends to decrease the validity of the researchers conclusions. There is more data here than has been analyzed and interpreted.

Review – Confidence index ranking:

Moderate

Author: Spiegelman JR, Hanson GD, Lazarus A, Bennett RJ, Lippmann M, Albert RE	**Internal ID: 205**
Title: Effect of acute sulfur dioxide exposure on bronchial clearance in the donkey.	
Year: 1968	
Abstract: The effects of 30-minute sulfur dioxide (SO_2) inhalation exposures on the bronchial clearance of radioactive monodisperse ferric oxide particles were studied in three miniature donkeys. The SO_2 concentrations ranged from 26 to 713ppm. No impairment of bronchial clearance was observed at levels below 300ppm. Higher concentrations produced severe cough and either slowing or transient arrest of bronchial clearance.	
Objective: "This study describes the impairment of bronchial clearance in the miniature donkey produced by 30-min exposures to high concentrations of SO_2."	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
27-713ppm	**30min – multiple times over 3mos**	**Donkey**	**miniature**	**3.5-7yrs 175-225lbs**	**1M 2F**	**3**	**n/a**	**<300ppm**	**300ppm**

Observations:

After exposure to 300ppm: "there was slowing in the rate of lower lung clearance and an abnormally large wave of activity in the upper lung with a lag in the onset of clearance of approximately 1 ½ hours.

After exposure to 500ppm: "…clearance from the lower lung was abnormally slow and clearance from the upper lung was abnormally delayed."

"…no increase in the weighted average bronchial clearance time was observed at SO_2 exposures below 300ppm. The maximum effect was observed at SO_2 exposures ranging from 557 to 713ppm and was characterized by a sixfold increase in the average bronchial clearance time in the three donkeys. Recovery was reasonably complete within a few days even after exposures which produced marked impairment."

*Statistically significant

Author's Conclusions:

"Normal bronchial clearance in miniature donkeys is highly reproducible and impairment after high-level exposures to SO_2 is readily apparent. With impaired clearance, all three donkeys showed an abnormally large initial wave of activity in the upper lung field, and a considerable lag in the onset of clearance."

Review – Assessment:

A. Test animals:	-✐ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ±✐ Are animal characteristics adequately recorded and appropriate for the objective of the study? -a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.*

	+-b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✏ Were the chosen exposure levels appropriate to investigate the primary objective? -✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? -✏ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? -✏ Was evidence provided to justify the dosing regime? -✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✏ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* ±✏ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +/-✏ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* +✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined.

	☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* ±✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? ±✎ Is the control regime appropriate? -✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? -✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +-✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✎ Was individual data reported for each test animal?
G. Data analysis:	-✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? ±✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? ±✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings demonstrate that donkeys have a rather large threshold in terms of SO_2 exposure (300ppm) before negative respiratory effects are observed. It must be noted that though donkeys were exposed nasally, nasal catheters were used to prevent SO_2 absorption by the nasal passages, a wise precaution. From this study, it appears that even donkeys may represent a useful animal model for study of SO_2 exposures.

Confidence index:

Only three test animals. Did try to limit confounding variables by having each donkey serve as its own internal control, also used exposure conditions that would limit absorption of SO_2 in the nasal passages. Used a single endpoint for determining bronchial clearance – measured amount of an aerosolized radioactive tag remaining in the chest at 2 different positions following co-inhalation with SO_2. Exposure conditions were fairly well defined, as were assessment methods – study should be reproducible. Varying doses given to each donkey make it a little difficult to assign significance to the data.

Review – Confidence index ranking:

Low

Author: Vai, F., Fournier, M., Lafuma, J., Touaty, E., Pariente, R.	**Internal ID:** 206
Title: SO_2-Induced Bronchopathy in the Rat: Abnormal Permeability of the Bronchial Epithelium *In Vivo* and *In Vitro* after Anatomic Recovery	
Year: 1980	
Abstract: The effects of different SO_2 exposures on the uptake and transport of exogenous proteins by the trachobronchial epithelium were investigated *in vivo* and *in vitro* using explanted tissue from rat lungs. By optical and electron microscopy, modifications in ferritin uptake and transport were observed, depending on SO_2 exposure and on subsequent structural changes; these changes involved a considerable increase in mucosal permeability, both *in vivo* and *in vitro* affecting the epithelium of the trachea and main bronchi. Such an increase, although reduced, was observed 3 months after SO_2 exposure had been discontinued, at a time when the structure appeared normal. These findings suggested that SO_2-induced changes in upper respiratory epithelium may be responsible for long-term abnormal permeability and that such changes may contribute to the pathogenesis of chronic bronchopathy.	
Objective: The purpose of the present study was to investigate bronchial permeability in the rat, in vivo and in vitro using tracheobronchial explants, immediately after different SO_2 exposures and 3 months after SO_2 exposure had been discontinued.	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
600 ppm	30-100 hours	Rats	Sprague-Dawley	**NA**	Both	47	NA	No observed adverse effect level	Lowest observed adverse effect level

Observations:

Depending on SO_2 exposure and on subsequent structural changes; these changes involved a considerable increase in mucosal permeability, both *in vivo* and *in vitro* affecting the epithelium of the trachea and main bronchi. Such an increase, although reduced, was observed 3 months after SO_2 exposure had been discontinued, at a time when the structure appeared normal.
*Statistically significant

Author's Conclusions:

These findings suggested that SO_2-induced changes in upper respiratory epithelium may be responsible for long-term abnormal permeability and that such changes may contribute to the pathogenesis of chronic bronchopathy. Prolonged SO_2 exposure does not increase the subsequent anatomic damage; this fact support the belief that for an intermittent SO_2 exposure of 600 ppm 2 to 5 h a day, the bronchal mucosa could reach a steady state, an equilibrium between injury and repair.

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (+) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+/-) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective?

	(+/-) Was evidence provided to justify the dosing regime? (-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+/-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+/-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+/-) Was the assessment thorough (*i.e.*, information pertaining to the clinical

	nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+/-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(N/A) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (N/A) Did the investigator appropriately interpret the statistics? (N/A) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+/-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study looked at the effect of SO_2 exposure on bronchial permeability in the rat 3 months after exposure had been discontinued. The study used tracheobronchial explants to show that the selectivity of the bronchial lymphoepithelium in uptake and transport of ferritin disappears. This abnormal permeability may contribute to the pathogenesis of chronic bronchopathy. The fact that an abnormal permeability of the bronchial lymphoepithelium existed in the rats three months after exposure indicates that SO_2 of acute exposure can have longer lasting effects.

Confidence index:

The study was conducted according to guidelines and the number of animals used increases the confidence placed on the results. However, the lack of reporting of the statistical methods used and health and diet of the rats diminishes the confidence of the study. Furthermore, exposure conditions were not well defined, and assessment methods were mostly based on morphological data – very subjective (scored as + or 0). Consequently, the overall study may not be reproducible. Functional data was also based on optical and electron microscopy to visualize ferritin uptake in explanted lung – again scored at + or 0 and very subjective. Despite methodological limitations, the anatomical abnormalities observed during the study were in agreement with previously published works.

Review – Confidence index ranking:

Moderate

Author: Ukai K	**Internal ID: 207**
Title: Effect of SO_2 on the pathogenesis of viral upper respiratory infection in mice.	
Year: 1977	
Abstract: n/a	
Objective: "The present study was designed to determine the effects of constant exposure of nasal mucus membranes of mice to low levels of SO2 on the pathogenesis of influenza virus infection in these animals."	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0.03-0.1ppm	**4 weeks**	**Mice**	**Japanese DS**	**4-5wks 15-20g**	**Male**	**n/a**	**Healthy or influenza**	**n/a**	**0.03-1.0ppm**

Observations:

"Histopathological changes in nasal tissues showed that inflammatory response was more rapid and more severe, and regeneration was initiated sooner, in these mice (virus infected and SO_2 exposed) than in virus inoculated controls. The HI titer also developed more rapidly and reach higher levels in mice exposed to SO_2 plus virus than in mice receiving virus alone, and antibody to the virus appeared earlier in the exposed mice. Animals which were exposed to SO_2 and were not given the virus showed a sixfold increase in the number of goblet cells in the nasal respiratory epithelial cells, though the olfactory epithelial cells were apparently unaffected."
*Statistically significant

Author's Conclusions:

"The results suggest that increased severity of influenza infection after continued exposure to low levels of SO_2 resulted from a progressive alteration of the nasal mucus membranes by SO_2 which then allowed rapid destructive proliferation of subsequently introduced virus. This destruction of nasal tissues would eliminate a major defensive barrier against lower tract disease."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.*

	+c) Sex +/-d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation ☐ *Guideline: Below 20% variation* +f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ±✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* +✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* +✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided?

	-✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? +✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? ±✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? -✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	-✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? ±✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

A population of mice (n=approx 30) were chronically exposed to SO_2, and then some were inoculated with a mouse-adapted influenza virus to determine how the upper nasal tissue reacts to this kind of exposure. The results demonstrate that the nasal tissue reacts similarly to the tracheal epithelia to low levels of SO_2 and as the bronchial epithelia does to heavy concentrations of SO_2. This does indicate that the upper respiratory tract, the area that first contacts SO_2, is affected by low level, chronic exposure. If these findings can be extrapolated to humans, it could have implications that are in situations where they are exposed to SO2 in significant amounts, because if they become ill, the effects of SO2 exposure could become exacerbated.

Confidence index:

Sufficient sample size to assess the effects of SO_2 on viral infection. Exposure conditions and assessment methods are fairly well defined, with the exception of actual exposure dose – study should be easily reproducible. Multiple parameters were studied – histopathology was extensive. However, the results presented were incomplete, in many case the SO_2 alone data was not discussed at all and only comparisons between virus and virus + SO_2 were made, making it difficult to interpret effects of SO_2 alone versus SO_2 + virus – relied too heavily on what was in the literature to perform a thorough assessment. Lack of information about equilibration of the chamber before testing, lack of information about the exact number of animals ("approximately 30"), and lack of detailed statistical analyses on the results detract from the confidence in the work.

Review – Confidence index ranking:

Moderate to low

Author: Weiss, K., Weiss, H.	**Internal ID:**208
Title: Increased Lung Compliance in Mice exposed to Sulfur Dioxide	
Year: 1976	
Abstract: Mice exposed 6-9 days to 40 ppm SO_2 lost 21% of body weight, but static lung compliance increased 37 % ($p<0.01$). slopes of saline volume-pressure cures increased 8%((non-sig) pointing to a decrease in surface tension rather than tissue elasticity. Alveolar stability, lung weight and lung wet/dry ratio decreased 2 to 6%, whereas trapped gas increased 17 %, but none were statistically significant.	
Objective: To document experiments that showed a significant increase in lung compliance with moderate concentrations of SO_2.	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
40 ppm	6-9 days	Mice	CD-1	Adult	Female	50	Free of pulmonary infectioins	No observed adverse effect level	Lowest observed adverse effect level

Observations:

Mice exposed 6-9 days to 40 ppm SO_2 lost 21% of body weight, but static lung compliance increased 37 % ($p<0.01$)..
*Statistically significant

Author's Conclusions:

The increase in compliance is compatible with the absence of edema. It appears due primarily to a decrease in surface tension rather than tissue elasticity. This is also the conclusion reached by others working with lung irritants. However, since alveolar stability was essentially unchanged, neither the surfactant concentration nor the geometry of individual alveoli appears to have been greatly altered, but the 17% increase in trapped gas (not sig.) may indicated the recruitment of normally closed alveoli.

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study?

	(+) a) Specie; mouse *Guideline: Rat, or justification for use of another mammalian specie.* **(+) b) Age +** *Guideline: Adult, i.e.,8-12 weeks.* **(+) c) Sex; +** **(+) d) Pre-test health +** *Guideline: Females should be nulliparous and non-pregnant.* **(+) e) Weight variation +** *Guideline: Below 20% variation* **(+) f) Source +** *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	**(+/-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +** **(-) Was there a gradient of exposure levels? -** *Guideline: Three exposure levels in addition to control.* **(+) Were the chosen exposure levels appropriate to investigate the primary objective? +** **(+/-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +** **(+) Were both nominal and actual exposure concentrations recorded? +** **(+) Was the duration of exposure appropriate to investigate the primary objective? +** **(-) Was the duration of exposure precisely defined? -** **(N/A) If exposure chambers were employed, were animals exposed during the equilibration period? NA** *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(+) Was the mode of administration appropriate? +** **(+) Was the frequency of administration appropriate to investigate the primary objective? +** **(+) Was evidence provided to justify the dosing regime? +** **(+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously? +**
C. Housing/Feeding:	**(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? +** *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%*

	(-) Was the number of animals grouped in a single chamber stated? - *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? NA *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? - *Guideline: Conventional lab diet* (+/-) Was the amount and feeding schedule specified? + *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+/-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. + *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+) Was a full description of the monitoring devices provided? + (+/-) What was the sensitivity of the monitoring devices employed? +- 15%
E. Procedural:	(N/A) Was the acclimation period specified? Was it of acceptable duration? NA *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? - (+/-) Were test animals randomly assigned to exposure groups? NA (-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? + (+) Is the control regime appropriate? + (N/A) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? + (+/-) Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? + (+) Were accepted assessment methods employed? + (+) Were assessment measures sufficiently sensitive? + (-) Was the nature and severity of symptoms graded against an explicitly defined scale? - (+/-) Did qualified personnel conduct the exams? + (+) Was the timing and frequency of assessment logical? +

	(+) Was raw data provided to permit the reader to arrive at his/her own conclusions? + (-) Was individual data reported for each test animal? -
G. Data analysis:	(N/A) Are the statistical methods used appropriate? NA (-) Are confidence intervals reported? - (N/A) Did the investigator appropriately interpret the statistics? NA (N/A) Are the observed trends statistically significant? NA
H. Interpretations:	(+) Was the original objective addressed? + (-) Are the results novel? - (+) Was the design of the study appropriate to investigate the primary objective? + (+) Were assumptions reasonable? + (+) Are the methods reproducible? + (-) Did either the study design or the statistical analysis limit confounding variables? - (+/-) Do the conclusions make biological sense? + (N/A) Are the extrapolations reasonable? + (+) Is the paper in a peer-reviewed journal? + (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study looked at the effect of SO_2 on lung compliance in mice. The only statistically significant finding was that static lung compliance increased by 37% after SO_2 exposure ($p<0.01$). Mice were exposed to high levels (40 ppm) of SO2 and then lung compliance was evaluated against control groups. It was determined that there was a significant (21%) weight loss in the experimental group, and an increase of 37 % in lung compliance. While the study does have relevance to human response to SO_2 concentrations that are higher than found in "normal" air pollution, the toxicological significance of the findings are not fully developed.

Confidence index:

Sufficient sample size to provide statistically relevant data. Exposure conditions and assessment methods were not well defined. For example, lung compliance and alveolar stability were measured from static volume pressure curves from lungs *in situ* – no experimental details as to how this is achieved. Additional parameter should have been looked at (morphologic data would have been easy) – extrapolation of increased lung compliance to mean absence of edema when lung weight and trapped gas were not significantly different is unwarranted. No mechanism for why SO_2 should increase lung compliance. Furthermore, while the population size, strain, and feeding are well within guidelines; the lack of reporting the statistical tests used, the variation, or loose definition of exposure time (6-9) days lowers the confidence in the study.

Review – Confidence index ranking:

Low

Author: Watson AY. Brain JD.

Internal ID: 209

Title: The effect of SO_2 on the uptake of particles by mouse bronchial epithelium.

Year: 1980

Abstract: In three experiments, we have explored the uptake and transport of colloidal gold (Au) and iron oxide (Fe_2O_3) by normal and SO_2-injured bronchial epithelium. In the first experiment, mice were exposed to a 2-hr aerosol of Au; in the second experiment, mice were exposed to SO_2 (250 ppm) for 3 hr, then to Au; and in the third experiment, mice inhaled SO_2 and then were exposed to Fe_2O_3. In each experiment, animals were killed at 0 hr, 1 day, and 4 days postexposure. From each animal, samples of large airways were examined by electron microscopy, and the cell type and particle location were recorded. Au was not readily taken up by the bronchial epithelium in that only two cells out of 1162 contained particles. No Au was seen in the submucosa. Exposure to SO_2 produced a mild, but nonnecrotic response characterized by an increase in mucus, vacuolated cells, detached cilia, and an influx of polymorphonuclear leukocytes. SO_2 did not affect the uptake of Au; 2 out of 1177 epithelial cells contained Au. However, gold did reach the submucosa. Results from the SO_2 plus Fe_2O_3 experiment were compared to a previous study of similar design in which mice were exposed only to Fe_2O_3. SO_2 increased the uptake of iron and affected the distribution of intracellular iron among the different cell types. It is possible that increased iron uptake is due to either injury or to changes in particle solubility produced by the SO_2.

Objective: "Our purpose was to quantify the extent of normal particle uptake in injured airway epithelium and compare it to that seen in the normal airway."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
250ppm	3hr	Mice	CD-1	n/a 20-25g	male	9 x 3 = 27 10 x 1 = 10	n/a healthy?	n/a	250ppm

Observations:

Exposure to colloidal gold and SO_2 resulted in many cells becoming vacuolated, an influx of polymorphonuclear leukocytes, and some animals had an increased thickness of the mucus layer. No gold particles were able to penetrate epithelial cells, even in the presence of SO_2.

Exposure to iron oxide and SO_2 produced similar effects to those above, including vacuolated cells, increased mucus, detached cilia, and neutrophils were observed. However, duration of response appeared longer in the iron oxide exposed group than the gold exposed group.

In comparison with a previous experiment of iron oxide uptake where there were no exposures to SO_2, iron oxide uptake was significantly increased in all cell types observed when co-exposed to SO_2 at each time point measured (0, 1 day, 4 days).

There was no difference in the amount of sulfur colloid aerosol remaining in either the SO_2-exposed or unexposed animals.

*Statistically significant

Author's Conclusions:

"By exposing animals to SO_2, we produced a nonlethal insult to airway epithelial cells. Although no changes in the epithelial response were detectable, gold particles do, but infrequently, reach the submucosa by an unknown route. SO_2 exposure does increase the uptake of iron by the epithelium. This increase may be due to an increase in the amount of soluble iron present in the mucus rather than a result of cell injury. We conclude that an insulted but intact epithelium is still reasonably effective in excluding particles. It also appears that properties of a particle are important in determining the response of injured as well as normal epithelium."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* +f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?

C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* ±✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? ±✎ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? -✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?

G. Data analysis:	+✓ Are the statistical methods used appropriate? -✓ Are confidence intervals reported? +✓ Did the investigator appropriately interpret the statistics? +✓ Are the observed trends statistically significant?
H. Interpretations:	+✓ Was the original objective addressed? +✓ Are the results novel? +✓ Was the design of the study appropriate to investigate the primary objective? +✓ Were assumptions reasonable? +✓ Are the methods reproducible? +✓ Did either the study design or the statistical analysis limit confounding variables? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings demonstrate that SO_2 can act as an insult to mouse bronchial epithelium, and can produce a number of mild symptoms. The purpose of this study was to assess the extent of particle transport in injured epithelial cells; SO_2 was used as an agent to produce airway injury. While SO_2 did not increase the uptake of gold, it did increase uptake of iron. These results suggest that SO_2 can have clinically significant effects on particle uptake in the airways, but that the effects can vary with the particle in question. However, the conclusion that injured but intact epithelium is still an effective barrier to particle entrance into cells is important, because the effect of airborne irritants on immune-compromised individuals could have a bearing as these populations may be more susceptible to lower chronic doses of chemical and particle densities – warrants further study into whether this is due to injury of particle solubility produced by SO_2.

Confidence index:

Insufficient sample size for statistical relevance – none was attempted. Experimental design was well defined and should be easily reproducible. Endpoints used electron microscopy to assess location and quantify particles – can be subjective, especially when assigning cell classes. The short time frame of post-exposure observation (max 4 days), the lack of information about test animals pre-exposure health, living conditions, and age lower the confidence in an otherwise well-done study. Novel result that SO_2 can increase the uptake of iron and affected the distribution of intracellular iron among the different cell types – warrants further study into whether this is due to injury of particle solubility produced by SO_2.

Review – Confidence index ranking:

Moderate to high

Author: Pariente, R. **Internal ID:** 210

Title: Lung Toxicity of Some Atmospheric Pollutants

Year: 1980

Abstract: Using an electron-dense tracer, i.e. ferritin, permeability of the tracheobronchial epithelium was studied in vivo an in vitro in rats exposed to SO_2. These experiments were performed with various concentrations of SO_2 and the most interesting results concerned animals left for three months in ambient atmosphere after SO_2 exposure had been stopped. Whereas anatomic recovery was totally achieved, it was observed an increased transepithelial permeability when the initial exposure exceeded 400 ppm of SO_2. When comparing these results with those obtained from epidemiologic studies, one must underline the difficult to assess the toxicity of atmospheric pollutants.

Objective: To assess on one hand the highest exposures in terms of concentrations without lethal effects and on the other had long-term exposures that would induce bronchial injuries similar to chronic obstructive lung disease ones.

To assess bronchial anatomic and physiologic recovery in rats after a long-term exposure (SO_2 600 ppm, 100 hr cumulated).

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
600 ppm 1000 ppm	up to 100 hours 4hrs	rats	unknown	unknown	unknown	unknown	unknown	N/A	400 ppm

Observations:

A four hour SO_2 exposure (1000 ppm) was not lethal and induced an acute, proximal and ulcerative bronchitis, with bleedings of the rhinopharynx.

Cumulative exposures (600ppm; total: 100hrs) led to chronic and proximal tracheobronchial injuries. Anatomic recovery was totally achieved.

The permeability of the tracheobronchial epithelium in vivo or in vitro was considerably increased along the whole bronchial tree when the intial exposure exceeded 400 ppm of SO_2.

*Statistically significant

Author's Conclusions:

"SO_2 exposure at 1000 ppm for 4 hours was not lethal. Cumulative exposure induced some but not all bronchial injuries similar to those seen with chronic obstructive lung diseases. These injuries did not worsen along with the exposure suggesting a dynamic equilibrium between injury and repair."

"Both data from experimental and epidemiological researches may improve our knowledge of the deleterious effects of some oxidizers and other atmospheric pollutants. However, as long as the biochemical mechanisms leading to anatomical and/or physiological damages will remain unknown, we shall only raise hypothesis concerning the direct or indirect connections between a toxic and the injuries observed."

Review – Assessment:

A. Test animals:	(+/-)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+/-)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+/-) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+/-)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+/-)Was the mode of administration appropriate?

	(+/-)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+/-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water May be withheld optionally.*
D. Equipment:	(+/-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+/-)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random Assignment) (+/-) Was a control group assayed? (+/-) Is the control regime appropriate?

	(+)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined? (+/-)Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+/-)Were accepted assessment methods employed? (+/-)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+/-) Are the statistical methods used appropriate? (-)Are confidence intervals reported? (+/-)Did the investigator appropriately interpret the statistics? (+/-)Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+/-)Was the design of the study appropriate to investigate the primary objective? (+/-)Were assumptions reasonable? (+/-)Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+/-) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The purpose of the study was to identify the highest dose of SO_2 that was non lethal and to assess bronchial anatomic and physiologic recovery in rats after a long-term exposure. These objectives were met and it was found that an increase in the permeability of the tracheobronchial epithelium occurred after long-term exposure to SO_2. However, no mention of sample size is made, casting doubt on the reliability of results.

Confidence index:

This paper basically just contains a discussion of the results of an experiment conducted by these authors. The actual experimental information is included in another paper. Without knowing any of the details of the study design, experimental protocol, etc.,it is impossible to review this paper.

No statistical analysis is included on the data – in fact no data was presented per se, only general trends observed in terms of anatomical and physiological effects. The exposure conditions and assessment methods were not well defined – this study would be impossible to reproduce.

Review – Confidence index ranking:

Low

DRAFT

Author: Wang AL. Blackford TL. Lee LY

Internal ID: 211

Title: Vagal bronchopulmonary C-fibers and acute ventilatory response to inhaled irritants.

Year: 1996

Abstract: Experiments were carried out in anesthetized Sprague-Dawley rats to determine the role of vagal bronchopulmonary C-fiber afferents in regulating the respiratory responses to inhaled irritants. Spontaneous inhalation of 2 tidal breaths of a known airway irritant (sulfur dioxide, 0.5%; ammonia, 1%; cigarette smoke, 50%) into the lower airways invariably elicited an immediate and transient inhibitory effect on breathing, characterized by apnea or bradypnea and accompanied by bradycardia, which lasted for 3-8 breaths. A delayed hyperpnea was also induced by inhalation of cigarette smoke, but not by sulfur dioxide or ammonia. After perineural capsaicin treatment of both cervical vagi to selectively block the conduction of capsaicin-sensitive C-fibers, these inhaled irritants no longer evoked any inhibitory effect on breathing; conversely, an augmented inspiration was triggered within the first 3 breaths from the onset of cigarette smoke inhalation in > 85% of the rats studied, but after a delay of several breaths following inhalation of ammonia or sulfur dioxide in only 30% of the rats. The augmented breaths were completely abolished when both cervical vagi were cooled to 6-7 degrees C. Bilateral vagotomy eliminated all the immediate responses to these irritants. These results suggest that both vagal C-fiber endings and irritant receptors in the airways are activated by these inhaled irritants, but the more dominant and consistent inhibitory effect on breathing is elicited primarily by stimulation of C-fiber afferents.

Objective: "The purposes of this study were: (1) to describe the acute respiratory responses to brief exposure (spontaneous inhalation of 2 tidal breaths) to these irritant gases; and (2) to determine whether the responses were mediated through bronchopulmonary reflexes, and if so, to identify the types of sensory afferents involved in evoking the acute effects of these irritants on breathing."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0.5% SO_2	**2 tidal breaths**	**Rat**	**Sprague-Dawley**	**Young 439±8g**	**Male & female**	**31**	**healthy**		

Observations:

"Immediately upon the first breath of SO_2 inhalation, an inhibitory effect on breathing, resulting primarily from a prolongation of TE (expiratory time) was consistently elicitied, and the bradypnea last for 3-8 breaths before returning to control… VI (inspiratory volume) was markedly reduced from a baseline of 173±18ml/min to 91±14ml/min ($p<0.001$. n=11) after the SO_2 inhalation. Accompanying the bradypnea, a mild decrease in HR (heart rate) from a baseline of 310±9beats/min to 281±13 beats/min (averaged over 10-sec periods) ($p<0.05$, n = 11), though ABP (arterial blood pressure) did not change significantly as a group. After the perineural capsaicin treatment, the same SO_2 inhalation challenge no loner evoked any inhibitory effect on breathing in the same animals. Conversely, in four of the eleven rats having received the capsaicin treatment, an augmented breath was triggered 6-8 breaths after the SO_2 inhalation. After bilateral vagotomy, inhalation of SO_2 no longer induced any change in VT, f or VI."

*Statistically significant

Author's Conclusions:

"Results obtained from this study clearly show that inhalation of two tidal breaths of common inhaled irritants such as sulfur dioxide, ammonia, and cigarette smoke into the lower airways evoked an immediate inhibitory effect on breathing in anesthetized rats. The bradypneic response delays and reduces the inhalation of these irritants and therefore serves a protective function. Since this response was initiated consistently upon the very first breath of irritant inhalation and was completely abolished by bilateral vagotomy, we conclude that vagal sensory receptors in the lungs and airways are responsible for triggering this respiratory reflex response."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* ±c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? -✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? ±✎ Was evidence provided to justify the dosing regime? +/-✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?

C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* -✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? -✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? -✎ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +/-✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? -✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?

G. Data analysis:	+/-✓ Are the statistical methods used appropriate? ±✓ Are confidence intervals reported? +✓ Did the investigator appropriately interpret the statistics? +✓ Are the observed trends statistically significant?
H. Interpretations:	+✓ Was the original objective addressed? +✓ Are the results novel? +✓ Was the design of the study appropriate to investigate the primary objective? +✓ Were assumptions reasonable? +✓ Are the methods reproducible? +✓ Did either the study design or the statistical analysis limit confounding variables? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings demonstrate that the mechanism of respiratory response to inhaled irritants is mediated by vagal nerves. Upon severing of these nerves, irritant induced bradypnea and inspiratory volume decreases were no longer observed. Perineural capsaicin treatment also removed any inhibitory respiratory effects of irritant inhalation. The inhibitory responses seen appear to be a defense mechanism to decrease the amount of irritant inhaled. Results from this study suggest that vagal C-fiber endings and irritant receptors in the airways are activated by these inhaled irritants, but the more dominant and consistent inhibitory effect on breathing is elicited primarily by stimulation of C-fiber afferents. Stimulatory effects of SO_2 on airway afferents have been previously documented, but this study permitted description of respiratory responses to brief exposures to irritant gases and determined whether responses were mediated through bronchopulmonary reflexes.

Confidence index:

Study helps to identify the mechanism by which inhalation of SO_2 causes respiratory defense reflexes such as apnea and bradynea. Although statistical analysis was addressed in methods, none was shown. Sufficient sample size, with each rat also serving as its own internal control, same sex and similar weight and age – tried to limit confounding variables. In addition, no mention is made of pre-trial conditions, acclimation period of feeding and watering. It is not clear how many animals were grouped in a chamber together, or how grouping was accomplished. Finally, duration of exposure is not precisely defined and while nominal values of SO_2 are reported, actual values are not. Experimental design was well defined – study should be easily reproducible.

Review – Confidence index ranking:

Moderate

Author: Vanjonack, WJ. And Johnson, HD.	**Internal ID:** 212
Title: Effect of Sulphur Dioxide and Heat Stressors on Plasma Glucocorticoids and Thyroxine Levels in Mice	
Year: 1974	
Abstract: The objective of this study was to determine acute effects of SO_2 and simultaneous exposure to SO_2 and heat on plasma glucocorticoids and thyroxine levels. Male mice (160) were divided into four treatment groups: control, heat, SO_2, SO_2 and heat. These groups were further divided into four time exposures, 30 min, 1, 12 and 24 hours. The effect of heat and SO_2 as compared to heat or SO_2 alone appears to be a more rapid and prolonged elevation of glucocorticoids, while thyroxine levels showed a more rapid depression compared to heat and a greater depression as compared to the SO_2 treatment group.	
Objective: To determine acute effects of SO_2 and simultaneous exposure to SO_2 and heat on plasma glucocorticoids and thyroxine levels.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
40 ppm	0.5 to 24 hours	mice	Charles River CD-1	60 days	male	160	healthy	unknown	unknown

Observations:

The effect of SO_2 on plasma thyroxine levels showed a time dependent decrease that was statistically significant at 12 and 24 hr exposure times*. In contrast, there was a time dependent increase in plasma glucocorticoids that was significant at 1 and 12 hours*. SO_2 also caused a significant decrease in rectal temperature at 1 and 12 hour exposure times*. These effects were exacerbated by addition of heat.
*Statistically significant

Author's Conclusions:

"The effect of heat and SO_2, as compared to heat or SO_2 alone, appears to be a more rapid and prolonged response of glucocorticoid, while thyroxine levels showed a more rapid depression as compared to the heat treatment group and greater depression as compared to the SO_2 treatment group."

"This data suggests that mice exposed to SO_2 decreased their ability to thermoregulate."

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.*

	Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex (+) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation *Guideline: Below 20% variation* (+) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(+/-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+/-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+/-) Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers

	appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+/-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (+) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (+) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (+/-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+/-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+/-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+/-) Was the nature and severity of symptoms graded against an explicitly

	defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-). Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+). Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study looked at the effect of SO_2 on plasma glucocorticoids and thyroxine levels to assess its effects on endocrine function. Results from this study indicate that heat and SO_2 appear to bring about a more rapid and prolonged elevation of glucocorticoids, while thyroxine levels showed a more rapid depression compared to heat and a greater depression as compared to the SO_2 treatment group. This is one in very few studies that have examined the endocrine response of animals simultaneously exposed to chemical and environmental heat stressors.

Confidence index:

Sufficient sample size to generate statistically significant data. Some initial trends observed suggesting an inverse relationship between glucocorticoids and thyroxine, but by 24 hr levels returned to normal. Warrants more extensive dose and time response before any conclusions or extrapolations can be made about SO_2 effects on endocrine function. No attempt to explain the results with exception of thermoregulation. The investigators relay information about animal subjects, but do not describe the exposure chamber, nor make reference to the sensitivity of monitoring devices. The period of acclimation was not mentioned, nor was information about how randomization of subjects was accomplished.

Review – Confidence index ranking:

Moderate

Author: Oomichi S. Kita H.	**Internal ID: 213**

Title: Effect of air pollutants on ciliary activity of respiratory tract.

Year: 1974

Abstract: Activity of the cilia of the upper airway epithelium is on of the most important biological self-defense actions against the inhaled gases, fumes, and particles. However, there are only a few systematic reports on the influence of air pollutants on ciliary activity. The authors developed a new system to measure the ciliary activity as the beat rate in vitro, applying the phase-contrast microscope, photoelectric cell and oscillograph, and exposure chamber for the continuous ventilation of a gas of a certain concentration. For SO_2, NO_2, NO, HCHO, and O_3, as air pollutant gases, the influence these gases on the ciliary activity of the excised specimen of trachea of the guinea pig were experimentally studied. To compare the influence of each gas, the ideal of Half Reduction Time of ciliary activity by a gas of certain concentration was introduced and the time of each gas for 50ppm concentration was: SO_2, 4min; HCHO, 5min; NO_2, 10min; NO, 14min; and O_3, 20min. It can be assumed that the influence of the easily water-soluble gases such as SO_2 and HCHO on the ciliary activity is severer compared with the less water-soluble ones, NO_2 and O_2, as the latter gases pass over the mucous surface of the upper airway, without dissolving to the mucus layer.

Objective: "…the present authors studied the gas exposure influence on the ciliary activity with regard to the gas concentration and gas exposure time lapse and proposed the expression "Half Reduction Time of ciliary activity" to evaluate the noxiousness of a certain gas on the activity of tracheal cilia. This value is the time lapse from the gas exposure to the time point where the ciliary beat rate is reduced by half, being determined principally by the kind of gas and its concentration."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
15, 32, 58, 77ppm	**Varied ~2-16min**	**Guinea pig**	**Excised tracheae**	**n/a 365-850g**	**n/a**	**3-5/ treatment**	**n/a**	**n/a**	**15ppm**

Observations:

Decreases in ciliary activity progressed in dose-response fashion with increasing concentrations of SO_2. 'Half Reduction Time of ciliary activity' (HRT) for the varying levels of SO_2 are as follows: 15ppm – 13.0min; 32ppm – 6.5min; 58ppm – 3.5min; 77ppm – 2.0min.

*Statistically significant

Author's Conclusions:

"In the present paper, the Half Reduction Time of the ciliary activity by each gas at the concentration of 50ppm is: SO_2, 4min; HCHO, 5min; NO_2, 10min; NO, 14min; and O_3, 20min. Thus, gases of high water solubility, such as SO_2 and HCHO, have short Half Reduction Time, but on the contrary, gases of low water solubility, NO_2 and O_3, have a long Half Reaction Time."

Review – Assessment:

A. Test animals:	±✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? ±✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? -✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.*

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D. Equipment:	+/-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? ±✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* ±✎ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? ±✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? ±✎ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	±✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective?

	+✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings demonstrate that SO_2 has the greatest effect on inhibiting ciliary activity of the respiratory irritants studied. Further, it appears that water-soluble compounds are more effective at affecting respiration than those with low solubility. Also, the authors present a method of evaluating the severity of effect of respiratory noxae (the Half Reduction Time method).

Confidence index:

Sample size is not stated explicitly for individual trials, but is stated indirectly that it lies between 3-5 animals per treatment, which does not meet the review guidelines. This study does not make mention of possible confounders such as age, and gender of guinea pigs. Furthermore, feeding and housing schedule is not mentioned. No indication is given as to how guinea pigs were grouped, or if it was done randomly. Also, no statistical analyses are conducted on the data collected to evaluate the significance of the findings. A lot of preliminary expt were performed to ensure that ciliary activity was reproducible. Still, these are not expected to represent major problems for the study because of the clear dose-response patterns shown. Novel method utilizes a single endpoint of ciliary activity to assess noxiousness – method design allows for each trachea to serve as its own internal control. Experimental exposure conditions and assessment was well defined – should be easily reproducible. Finally, animal characteristics and their living conditions were not recorded thoroughly, but no apparent problems can be seen from the information provided.

Review – Confidence index ranking:

Moderate

Author: Petruzzi, S., Dell'Omo, G., Fiore, M., Chiarotti, F., Bignami, G., Alleva, E.	**Internal ID:** 214

Title: Behavioural disturbances in adult CD-1 mice and absence of effects on their offspring upon SO_2 exposure

Year: 1996

Abstract: Adult male and female CD-1 mice were exposed to different SO_2 concentrations (0,5,12, or 30 ppm) for 24 days, from 9 days before the formation of breeding pairs to pregnancy day 12-14. This exposure was near-continuous, covering about 80% of the total time indicated. The offspring of exposed dams were cross-fostered shortly after birth to dams not previously exposed. Videorecordings of the adult subjects' activities during the first hour after the start of exposure showed marked, acute transient behavioural effects such as increase of rearing and social interactions, which were more pronounced in males than in females. Subsequent activity tests on exposure days 3, 6, and 9 showed subacute effects including a dose-dependent decrease of grooming and an increase of digging as well as changes in chamber crossing and wall-rearing which were not dose-dependent; most of these effects were more pronounced in females than in males. Food and water consumption and body weight declined in a dose-dependent fashion only after the formation of breeding pairs, when consummatory responses were enhanced in the controls. Reproductive performance as well as postnatal somatic and neurobehavioural development of the offspring (the latter assessed by an observational test battery including eight reflexes and responses) were not affected by SO_2. Passive avoidance acquisition and retention at the young adult stage (60 days) and response changes produced by repeated apparatus exposure in non-reinforced animals (habituation) were similarly unaffected. Overall, the data indicate that SO_2 produces transient, acute behavioural disturbances and more subtle subacute response changes in adult mice which may be due, at least partly, to a functional interference with olfactory modulation of mouse behaviour. The absence of effects on reproductive performance and neurobehavioural development of the offspring suggests that the risk to the developing organism from gestational SO_2 exposure is low.

Objective: The aim of the present study was to evaluate the effects of SO_2 exposure before and during the first 2 weeks of pregnancy on the behaviour and reproductive performances of male and female CD-1 mice, and on the subsequent somatic and neurobehavioural development of their offspring (cross-fostered to non-exposed dams) until the young-adult stage.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
5, 12, 30 ppm	24 days – near continuous exposure	Mice	CD-1	adult	40 male 40 female	80	Non pregnant	unknown	5-12ppm

Observations:

Acute and subacute SO_2 effects in non-pregnant mice

Activity tests inside the exposure chamber (Exposure day (ED) 0

The data on response frequencies clearly indicate a concentration dependent increase in the frequency of rearing, sniffing and social interactions shortly after the start of SO_2 exposure. The response change was significantly larger in the 30ppm than in the 12ppm group in the initial 10 min exposure period $p<0.05$).These treatment effects were generally more marked in males than in females, with or without a significant concentrations dependence of the sex effect. Overall, the response changes resulted in a reduced duration of inactivity after the start of exposure, followed by a return to the pre-exposure baseline.

Activity tests outside the chambers (EDs 3, 6, 9)
In several instances, response changes produced by SO_2 differed depending on sex and day of exposure. There was a dose dependent decrease of grooming and an increase of digging as well as changes in chamber crossing and wall-rearing which were not dose dependent; most of these effects were more pronounced in females than in males.

Feeding and drinking, body weight, and reproductive performances
SO_2 did not affect either food or water intake until the pairing of males and females. Subsequently, the SO_2 mice showed a depression of food intake which was concentration dependent. The body weight of females was equally unaffected by SO_2 exposure until the formation of breeding pairs; SO_2 exposure at any concentration did not affect mating, proportion of successful pregnancies, litter size, sex ratio, or neonatal mortality.

Assessments in the offspring
Somatic and neurobehavioural development
(postnatal days 2-18).
Prenatal SO_2 exposure had no effect on birth weight or postnatal body weight gain.

Passive avoidance (PAJ) learning and retention (postnatal day 60)
Prenatal SO_2 did not affect passive avoidance learning and retention or response changes produced by repeated apparatus exposure in the non-reinforcement condition.

*Statistically significant

Author's Conclusions:

The results presented in this work show an interesting contrast between SO_2 production of acute and subacute behavioural changes in adult mice and absence of effects on reproductive performances and on somatic and neurobehavioural development of the offspring.

Review – Assessment:

A. Test animals:	(+)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+)b) Age

	Guideline: Adult, i.e.,8-12 weeks. (+)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (+) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(+)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+)Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%*

	(+) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (+) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (+)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water* *May be withheld optionally.*
D. Equipment:	(-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random Assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale?

	(+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (-)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (+)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

In this study, the behavioural disturbances in mice exposed to SO_2 and the effects on their offspring were studied. Acute SO_2 effects consisted mainly of response increases (particularly in the case of rearing, sniffing and social interactions) which were followed by a return to control baselines within 30-60 min.

Subacute effects observed in subsequent activity tests outside the exposure chambers on EDs 3-9 were variable. There appeared to be behavioural differences between males and females. The depression by SO_2 of feeding, drinking and body weight occurred several days after the start of exposure (specifically, right after pairing of males and females, when food and water intake increased) and subsequently became attenuated, but reappeared when food intake increased again as pregnancy progressed. SO_2 exposure had no effect on reproductive performances or somatic and neurobehavioural development in the offspring.

In this study the LOAEL for acute and subacute behavioural changes falls between 5 and 12 ppm which may be comparable to some occupational exposures. The analysis is very subjective and additional studies are needed to determine if the responses are due solely to the SO_2 exposure.

The relevance of the present result for the assessment of human risk may be confounded by the possibility that olfactory mechanisms are involved in the observed changes in mice. The role of olfactory functions in humans suggests a much lesser role of smell in behavior regulation than in the case of rodents.

Future studies should consider disturbances created by interference with sensory functions in the absence of significant damage or dysfunction as conventionally stated.

Confidence index:

This experiment exhibited sufficient sample size for statistical analysis and measured multiple parameters to assess SO_2 effects on neurobehavioral development of offspring and behavior of exposed mice. Exposure conditions and assessment methods were well defined and as such, the study should be easily reproducible. Housing and food were described, and climatic conditions described. However, the monitoring device and its accuracy were not mentioned, nor was the acclimation period mentioned. Pre-test conditions were also excluded. Preliminary experiments performed to find range of exposures, also used relevant levels of SO_2 (found in some work environments). It should be noted that the responses studied were behavioural disturbances that required close observation. Furthermore, the qualifications of the personnel conducting the experiments were not given. This study was novel in that it used cross-fostering procedures to discriminate between prenatal effects of maternal SO_2 exposure and postnatal maternal effects resulting from somatic or behavioral changes in the dam.

Review – Confidence index ranking:

High to moderate

Author: Amdur, MO.	**Internal ID:** 216
Title: The Physiological Response of Guinea Pigs to Atmospheric Pollutants	
Year: 1959	
Abstract: Changes in the mechanical behavior of the lungs were produced by 1 hour exposure of guinea pigs to low concentrations of irritant air pollutants. Sulfur dioxide, acetic acid, formaldehyde, formic acid and 0.8µ sulfuric acid produced bronchial constriction, whereas 2.5µ sulfuric acid resulted in obstruction of some of the larger air passages. As evaluated by the increase in pulmonary flow-resistance and the increase in work of respiration the most potent of the gaseous irritants was formic acid, followed by formaldehyde, acetic acid, and sulfur dioxide in that order. Extension of the exposure time indicated that the response to sulfur dioxide continued to increase during a 3 hour exposure, whereas prolonging the formaldehyde exposure to 4 hours indicated that the maximum response was produced in 1 hours. In the case of sulfur dioxide, acetic acid, and formaldehyde the response was greater when the gases were breathed through a tracheal cannula, thus by-passing the scrubbing effect of the upper respiratory tract. Using mixtures of sulfur dioxide and sulfuric acid mist, a synergistic toxic action was observed when the sulfuric acid particle size was 0.8µ but not when 2.5µ sulfuric acid was used. A potentiation of the response to sulfur dioxide was obtained with sodium chloride aerosol of 0.04u but not with 2.5µ, hence particle size appears to be an important factor in the potentiation of the response to irritant gases by aerosols. The possible reasons for this are discussed. When the four irritant gases used were studied in combination with 10 mg/m3 sodium chloride of 0.04µ diameter which was itself without effect on the animals, the response to sulfur dioxide and to formaldehyde was potentiated by the response after the termination of the exposure in the case of all four gases. The response to the combination of formaldehyde plus aerosol was greater than the response to the gas alone breathed through a tracheal cannula. The presence of a tracheal cannula used with the gas-aerosol mixture produced an increase exactly accounted for on the basis of the gas alone. At a given concentration of formaldehyde the response produced varied with the aerosol concentration. These experiments indicate that the increase of toxicity of a gas-aerosol mixture can be definitely related to the aerosol entity.	
Objective: To examine the physiological response of guinea pigs to atmospheric pollutants.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
2 to 1000 ppm	1to 3 hours	Guinea pigs	unknown	unknown	unknown	87 exposed plus controls	unknown	unknown	unknown

Observations:

Sulfur dioxide, acetic acid, formaldehyde, formic acid and 0.8µm sulfuric acid produced bronchial constriction, whereas 2.5µm sulfuric acid resulted in obstruction of some of the larger air passages. As evaluated by the increase in pulmonary flow-resistance and the increase in work of respiration the most potent of the gaseous irritants was formic acid, followed by formaldehyde, acetic acid, and sulfur dioxide in that order. Extension of the exposure time indicated that the response to sulfur dioxide continued to increase during a 3 hour exposure, whereas prolonging the formaldehyde exposure to 4 hours indicated that the maximum response was produced in 1 hours. In the case of sulfur dioxide, acetic acid, and formaldehyde the response was greater when the gases were breathed through a tracheal cannula, thus by-passing the scrubbing effect of the upper respiratory tract. Using mixtures of sulfur dioxide and sulfuric acid mist, a synergistic toxic action was observed when the sulfuric acid particle size was 0.8µm but not when 2.5µm sulfuric acid was used. A potentiation of the response to sulfur dioxide

was obtained with sodium chloride aerosol of 0.04μm but not with 2.5μm, hence particle size appears to be an important factor in the potentiation of the response to irritant gases by aerosols. The possible reasons for this are discussed. When the four irritant gases used were studied in combination with 10 mg/m^3 sodium chloride of 0.04μm diameter which was itself without effect on the animals, the response to sulfur dioxide and to formaldehyde was potentiated by the response after the termination of the exposure in the case of all four gases. The response to the combination of formaldehyde plus aerosol was greater than the response to the gas alone breathed through a tracheal cannula. The presence of a tracheal cannula used with the gas-aerosol mixture produced an increase exactly accounted for on the basis of the gas alone. At a given concentration of formaldehyde the response produced varied with the aerosol concentration.
*Statistically significant

Author's Conclusions:

"These experiments indicate that the increase of toxicity of a gas-aerosol mixture can be definitely related to the aerosol entity."

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* (+/-) b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* (+/-) c) Sex (+/-) d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation ☐ *Guideline: Below 20% variation* (+/-) f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+/-) Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* (+/-) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of

	appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+/-) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+/-) Was the mode of administration appropriate? (+/-) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+/-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration?

	☐ *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+/-) Was a control group assayed? (+/-) Is the control regime appropriate? (-) Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+/-) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(N/A) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (N/A) Did the investigator appropriately interpret the statistics? (N/A) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense?

	(N/A) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study reviews a variety of investigations done on SO_2 effect on guinea pigs. Results seem to suggest that the increase of toxicity of a gas-aerosol mixture can be definitely related to the aerosol entity. However, because the number of animals used in this experiment is not given, it is difficult to know whether statistical significance should be attached to the results. Furthermore, no attempt is made to predict the probable response of man from the data obtained with the guinea pig.

Confidence index:

This study seems most concerned with results obtained from previous studies. Consequently, much of the information related to exposure environment, feeding habits and acclimation is not given. Consequently, the study would not be easily reproducible. Specific information about the guinea pigs, such as numbers, gender, and age are not provided. Climatic conditions are not recorded and specific information about examination following exposure is not detailed. Because of the diversity of studies reviewed in this paper, it is difficult to follow; at times it is unclear that a separate study is being referred to.

This study does not make clear if animals were randomly exposed and if they were, what method of randomization was used. There are very few results regarding SO_2. The results of the mixtures are discussed in another study. Furthermore, there is no analysis of significance and the literature review is inadequate.

Review – Confidence index ranking:

Low

Author: Fiore M. Petruzzi S. Dell'Omo G. Alleva E.	**Internal ID: 217**

Title: Prenatal sulfur dioxide exposure induces changes in the behavior of adult male mice during agonistic encounters.

Year: 1998

Abstract: Sulfur dioxide (SO_2) is one of the most important pollutants of the western countries, responsible for several cardiopulmonary diseases in humans. SO_2 affects both young and adult people, causing low work productivity with social and economical costs extremely high for the communities. To test whether or not SO_2 produces changes in social and/or agonistic behavior of laboratory animals, outbred CD-1 male mice were prenatally exposed to different SO_2 concentrations (0, 5, 12, or 30 ppm) up to pregnancy day 14. At adulthood, following a 4-week isolation period, they underwent an aggressive encounter with CD-1 male opponents of the same age, body weight, and isolation condition (single 20-min session). The levels of several responses such as tail rattling, freezing, and defensive postures were reduced by the treatment, particularly during the initial period of the agonistic encounter, whereas offensive and attack behaviors were not significantly modified. In addition, rearing and social investigation increased. Overall, the present results indicate that prenatal SO_2 exposure can alter mouse social/agonistic behavior, apparently acting on the approach phase toward the opponent and suggestive of changes in the animals' capability to cope with threatening dangerous situations.

Objective: "To test whether or not SO_2 produces changes in social and/or agonistic behavior of laboratory animals."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0-30ppm	First 14 days of pregnancy (fetal exposure)	Mouse	CD-1	Adult	Male	Not explicitly stated (n=11-12 per trial)	Healthy prenatal	n/a	5ppm

Observations:

SO_2 exposure did not affect mating, proportion of successful pregnancies, litter size, sex ratio and neonatal mortality. Prenatal exposure modified the behavior of male mice during agonistic encounters. Body sniffing* and nonsocial activities were enhanced, whereas freezing, tail rattling and defensive behaviors decreased, particularly during the initial period of the agonistic encounter, whereas offensive and attack behaviors were not significantly modified. A significant interaction between exposure and repeated measures in the case of self-grooming duration* was observed, it is difficult to interpret because it appears mainly due to a single elevated value in the 5 ppm group in the last part of the test.

*Statistically significant

Author's Conclusions:

"In conclusion, our findings show that prenatal SO_2 effects alter the mouse behavior during aggressive encounters with a conspecific, acting on the animals' ability to cope with threatening situations, probably by small changes in the neural substrate of behavior. Furthermore, the possibility that rodent social behavior could be included in regulatory testing for human-made or natural hazardous agents remains open."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* +✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* +b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex +d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* +f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? +/-✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	+✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate?

	☐ *Guideline: Animals may be grouped by sex, or individually.* +✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* +✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✏ Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* +✏ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? +✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? +✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✏ Was individual data reported for each test animal?
G. Data analysis:	+✏ Are the statistical methods used appropriate? ±✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel?

	+✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study indicates that prenatal SO_2 exposure can alter mould social/agonistic behavior, apparently acting on the approach phase toward the opponent and suggestive of changes in the animals' capability to cope with threatening dangerous situations. Levels of various responses such as tail rattling and defensive postures appeared to decrease in a dose-dependent fashion. These results extend those demonstrated in a previous experiment by the same investigators, suggesting SO_2 effects on mouse social behavior without gross impairments in the animal's general neurobehavioral development. Results suggest that rodent social behavior be included in regulatory testing for natural hazardous agents.

Confidence index:

Although sample size was sufficient for statistically significant results, none were observed. Endpoints were changes in nonsocial and agonistic behavior – can be very subjective. The exposure conditions and assessment were provided in general terms could have been more defined, especially in terms of grading the behavioral responses. Study was limited to male mice only, also by prenatal exposure of SO_2, limits the direct action of SO_2 on animals' olfactory sensitivity.

Review – Confidence index ranking:

Moderate to high

Author: Cohen, H. J.
Drew, R. T.
Johnson, J. L.
Rajagopalan, K. V.

Internal ID: 218

Title: Molecular basis of the biological function of molybdenum: the relationship between sulfite oxidase and the acute toxicity of bisulfite and SO_2

Year: 1973

Abstract: The administration of tungsten to rats maintained on a low molybdenum diet resulted in a dose and time-dependent loss of sulfite oxidase (EC 1.8.3.1) and xanthine oxidase (EC 1.2.3.2) activities and hepatic molybdenum. These tungsten-treated animals appeared healthy, but were more susceptible to bisulfite toxicity. The median lethal dose for intraperitoneal bisulfite was found to be 181 mg of $NaHSO_3$ per kg for the animals deficient in sulfite oxidase, compared to 473 mg/kg for normal rats.

The survival time of rats exposed to SO_2 at concentrations of 590 ppm and higher was seen to be inversely related to the levels of SO_2. At 590 ppm and 925 ppm, control animals displayed symptoms of severe respiratory toxicity before death. At 2350 ppm of SO_2, death was preceded by seizures and prostration, symptoms observed with the systemic toxicity of injected bisulfite. At 590 ppm, animals deficient in sulfite oxidase were indistinguishable from control animals. However, at 925 ppm and 2350 ppm, the deficient animals displayed symptoms of systemic toxicity and had much shorter survival times. It is concluded that sulfite oxidase is instrumental in counteracting the toxic systemic effects of bisulfite, either injected or derived from respired SO_2. Respiratory death probably results from the toxicity of gaseous SO_2 before absorption as bisulfite and cannot be alleviated by sulfite oxidase. Sulfite oxidase does not appear to be inducible by either bisulfite or SO_2.

Objective: The authors' stated objective and identification of relevant experiments in documents reporting findings from multiple studies.

Using rats deficient in sulfite oxidase, to determine if this enzyme is involved in the oxidative metabolism of SO_2 and bisulfite which results in the toxification of these compounds.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
224 – 500,000 ppm	**4 hours for mortality or until death for survival time**	**rat**	**CD**	**unknown**	**male**	**208**	**unknown**		**224 ppm**

Observations:

Reported data

The survival time of rats exposed to SO_2 at concentrations of 590 ppm and higher was seen to be inversely related to the levels of SO_2. At 590 ppm and 925 ppm, control animals displayed symptoms of severe respiratory toxicity before death. At 2350 ppm of SO_2, death was preceded by seizures and prostration, symptoms observed with the systemic toxicity of injected bisulfite. At 590 ppm, animals deficient in sulfite oxidase were indistinguishable from control animals. However, at 925 ppm and 2350 ppm, the deficient animals displayed symptoms of systemic toxicity and had much shorter survival times.

*Statistically significant

Author's Conclusions:

Quotations of the author's primary conclusions
It is concluded that sulfite oxidase is instrumental in counteracting the toxic systemic effects of bisulfite, either injected or derived from respired SO_2. Respiratory death probably results from the toxicity of gaseous SO_2 before absorption as bisulfite and cannot be alleviated by sulfite oxidase. Sulfite oxidase does not appear to be inducible by either bisulfite or SO_2.

Review – Assessment

A. Test animals:	✎ Is the number of animals sufficient to produce statistically significant results? + ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? a) Specie + ☐ *Guideline: Rat, or justification for use of another mammalian specie.* b) Age unknown ☐ *Guideline: Adult, i.e.,8-12 weeks.* c) Sex male d) Pre-test health unknown ☐ *Guideline: Females should be nulliparous and non-pregnant.* e) Weight variation + ☐ *Guideline: Below 20% variation* f) Source + ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? - ✎ Was there a gradient of exposure levels? + ☐ *Guideline: Three exposure levels in addition to control.* ✎ Were the chosen exposure levels appropriate to investigate the primary objective? - ✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? - ✎ Were both nominal and actual exposure concentrations recorded? - ✎ Was the duration of exposure appropriate to investigate the primary

	objective? - ✎ Was the duration of exposure precisely defined? + ✎ If exposure chambers were employed, were animals exposed during the equilibration period? unknown ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* ✎ Was the mode of administration appropriate? + ✎ Was the frequency of administration appropriate to investigate the primary objective? - ✎ Was evidence provided to justify the dosing regime? - ✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously? -
C. Housing/Feeding:	✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? + ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* ✎ Was the number of animals grouped in a single chamber stated? - ☐ *Guideline: Groupings should permit clear observation of each animal.* ✎ Was the manner by which animals were grouped appropriate? + ☐ *Guideline: Animals may be grouped by sex, or individually.* ✎ Was the type and source of feed and water stated? Was it appropriate? + ☐ *Guideline: Conventional lab diet* ✎ Was the amount and feeding schedule specified? + ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. - ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* ✎ Was a full description of the monitoring devices provided? - ✎ What was the sensitivity of the monitoring devices employed? unknown
E. Procedural:	✎ Was the acclimation period specified? Was it of acceptable duration? n/a

	□ *Guideline: 5 days* ✏ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? - ✏ Were test animals randomly assigned to exposure groups? unknown ✏ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) - ✏ Was a control group assayed? + ✏ Is the control regime appropriate? + ✏ Was the period of observation following exposure appropriate? n/a □ *Guideline: Longer than 14 days.*
F. Data collection:	✏ Were appropriate endpoints examined? - ✏ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? - ✏ Were accepted assessment methods employed? + ✏ Were assessment measures sufficiently sensitive? - ✏ Was the nature and severity of symptoms graded against an explicitly defined scale? + ✏ Did qualified personnel conduct the exams? unknown ✏ Was the timing and frequency of assessment logical? n/a ✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? - ✏ Was individual data reported for each test animal? -
G. Data analysis:	✏ Are the statistical methods used appropriate? n/a ✏ Are confidence intervals reported? n/a ✏ Did the investigator appropriately interpret the statistics? n/a ✏ Are the observed trends statistically significant? n/a
H. Interpretations:	✏ Was the original objective addressed? - ✏ Are the results novel? - ✏ Was the design of the study appropriate to investigate the primary objective? - ✏ Were assumptions reasonable? - ✏ Are the methods reproducible? + ✏ Did either the study design or the statistical analysis limit confounding variables? - ✏ Do the conclusions make biological sense? -

	✎ Are the extrapolations reasonable? - ✎ Is the paper in a peer-reviewed journal? + ✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document? -

Review - Summary:

Discussion of findings:

Using rats deficient in sulfite oxidase, this study attempts to demonstrate that this enzyme is involved in the oxidative metabolism of SO_2 and bisulfite that results in the toxification of these compounds. Results indicate that sulfite oxidase is unable to protect against levels of inhaled SO_2 in the range of 500 ppm. At higher levels of SO_2, bisulfite concentration reaches high enough levels that systemic death precedes respiratory death. this study. At lower levels of sulfite oxidase, however, it is evident that the enzyme is involved in oxidative metabolism and subsequent detoxification of SO_2. However, there was no statistical significance in mortality between control animals and rats made deficient in this enzyme exposed to SO_2, although, there was an observable difference in survival time – mortality/survival was the only endpoint studied to arrive at their conclusion.

Confidence index:

Possible confounding factors such as age, gender and weight of rats are accounted for in the study. A control group was assayed and a gradient of exposure levels used. Furthermore, the exposure environment and pre-testing conditions were adequately recorded and mention made of the acclimation period. However, no mention was made of how animals were divided into test groups, of if animals were in the test chamber as equilibration was occurring.

Exposure doses were extremely high for assessment of the relationship between sulfite oxidase and SO_2 toxicity. Exposure conditions and assessment was very straightforward and easily reproducible. Endpoint of mortality was not particularly useful, as all animals died from exposure to SO_2, although there was some difference in survival time. This study might have benefited from monitoring other accepted assessment methods that are well characterized for SO_2.

Review – Confidence index ranking:

Low

Author: Bauer E	**Internal ID: 221**
Title: In vivo sampling and biochemistry of tracheobronchial secretions in the fowl. Influence of sulfur dioxide inhalation.	
Year: 1981	
Abstract: A new method for collecting tracheobronchial secretions from the anesthesized fowl has been developed. Sulfur dioxide inhalation leads to a highly significant increase of mucus output (wet weight), 320 % of control. Glycoprotein output is increased by only 50 %. Thus glycoprotein concentration is lowered to about 1/3 that of the control group. The carbohydrate pattern of the glycoproteins shows no significant alteration after sulfurdioxide treatment. Isoelectric focusing of the glycoprotein subunits derived from papain digestion shows no difference between the macromolecular pattern of the control and SO_2 group.	
Objective: To identify an animal model where it is easy to collect both normal samples of treacheobronchial secretions and "…to characterize the disease state by biochemical parameters from non-quantitatively collected aliquots of the airway secretions."	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
350-400ppm	**3hours single**	**Chicken (Gallus domesticus)**	**n/a**	**n/a 1.5-1.5kg**	**n/a**	**9**	**Healthy**	**n/a**	**350ppm**

Observations:

Sulfur dioxide inhalation leads to a highly significant increase of mucus output (wet weight), 320 % of control*. Glycoprotein output is increased by only 50 %. Thus glycoprotein concentration is lowered to about 1/3 that of the control group*. Significant decrease found for hexos-NH2, fucose, mannose and NANA.* The carbohydrate pattern of the glycoproteins shows no significant alteration after sulfur dioxide treatment. Isoelectric focusing of the glycoprotein subunits derived from papain digestion shows no difference between the macromolecular pattern of the control and SO_2 group.
*Statistically significant

Author's Conclusions:

"The animal model presented here is very suitable for the investigation of the action of agents on tracheobronchial secretion for four reasons: 1) low level irritation of the organ system during sample collection; 2) easy collection of samples; 3) large animal groups in one experiment; 4) sufficient biological material for all analytical work can be collected."

Review – Assessment:

A. Test animals:	±✎ Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie

	☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex +d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✏ Were the chosen exposure levels appropriate to investigate the primary objective? -✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? ±✏ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? -✏ Was evidence provided to justify the dosing regime? -✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✏ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✏ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✏ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*

D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? ±✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +/-✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? ±✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +/-✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal?

	+✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study evaluates *in vivo* sampling and biochemistry of tracheobronchial secretions in the fowl. Previous studies have studied inhalation of sulfurdioxide by dog and rat. However, this study uses an animal whereby normal secretions can be collected easily and the disease state can be characterized by biochemical parameters from non-quantitatively collected aliquots of airway secretions. The data shows that glycoprotein composition is significantly different in SO_2 exposed fowls compared to controls may provide a biomarker for characterizing bronchitis.

Confidence index:

Animal characteristics, gas generation, and exposure conditions are described poorly. Furthermore, the statistical analyses do not appear to be sufficient, as $P<0.1$ was the stated level of significance. This study did not describe all aspects of the materials and methodology. For example, information on breed, age and sex of animals were not given. Furthermore, pre-testing conditions including feeding regiment and acclimation period, are not listed, nor is climactic conditions during the exposure period. Furthermore, despite the apparently low numbers of test animals, raw data was not provided for each subject. Some of the negative data is not shown – no change in carbohydrate pattern and no difference in macromolecular pattern are only mentioned. Paucity of references so unable to determine if the results are novel or in accordance with what others have published.

Review – Confidence index ranking:

Low to Moderate

Author: Gunnison, A.F., Benton, A.W.,	**Internal ID:** 222
Title: Sulfur Dioxide: Sulfite Interaction with Mammalian Serum and Plasma	
Year: 1971	
Abstract: The chemistry of sulfite – bisulfite (the hydrate of sulfur dioxide) in mammalian plasma and serum was investigated in vitro and in vivo. The longevity of sulfite in contact with mammalian plasma and known components of blood was determined by adaptation of a colorimetric method for sulfite analysis. Evidence from all experiments indicated that, under physiological conditions, sulfite reacts reversibly with disulfide bonds present in the plasma resulting in formation of S-sulfonates (sulfitolysis). Free sulfite was not detected in plasma of rabbits immediately following exposure to approximately 25ppm SO_2, but there was good evidence for substantial elevation of plasma and serum S-sulfonate content. Reactivity of sulfite with plasma constituents may protect many body tissues from the insult of relatively high concentrations of sulfite and may facilitate prolonged exposure to very low levels of sulfite.	
Objective: The purpose of this study is to investigate the reactivity of sulfite or bisulfite (hereafter referred to simply as sulfite) with mammalian plasma and serum under physiological conditions.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
1.7, 3.6, and 5.2 nanomols of sulfite analyzed in the presence of serum	Varied	Rabbit	New Zealand	4-22 months	No info		No info	N/A	N/A
23.5 ppm SO_2	2 rabbits exposed continuously for 14 hours and 2 rabbits were exposed for 62 hours	Rabbit				4			

Observations:

Evidence from all experiments indicated that, under physiological conditions, sulfite reacts reversibly with disulfide bonds present in the plasma resulting in formation of S-sulfonates (sulfitlysis). Free sulfite was not detected in plasma of rabbits immediately following exposure to approximately 25 ppm SO_2 but there was good evidence for substantial elevation of plasma and serum S-sulfonate content. Reactivity of sulfite with plasma constituents may protect many body

tissues from the insult of relatively high concentrations of sulfite and may facilitate prolonged exposure to very low levels of sulfite.
*Statistically significant

Author's Conclusions:

The evidence presented supports the theory that sulfite which enters the bloodstream during exposure of a mammal to atmospheric SO_2 forms S-sulfonate groups with constituents of the plasma, probably exclusively by sulfitolysis of disulfide groups. The composite reaction involved apparently reaches a position of equilibrium with very close to 100% of the sulfite in the oxidized or sulfite form, provided that the capacity of the reactive groups of the plasma has not been exceeded by an excess of sulfite. This interaction quite probably affords protection of many tissues of the body from the insult of high concentrations of sulfite.

Review – Assessment:

A. Test animals:	(-)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (-)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels?

	(+)Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (+)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water* *May be withheld optionally.*
D. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease

	treatment, etc.) provided? (-) Were test animals randomly assigned to exposure groups? (N/A) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random Assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined? (-)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (+)Was individual data reported for each test animal?
G. Data analysis:	(N/A) Are the statistical methods used appropriate? (-)Are confidence intervals reported? (N/A)Did the investigator appropriately interpret the statistics? (N/A)Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (+)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (+/-)Were assumptions reasonable? (+)Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially

	bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The purpose of this study was to investigate the reactivity of sulfite or bisulfite with mammalian plasma and serum under physiological conditions. Results from this study suggest that, under physiological conditions, sulfite reacts reversibly with disulfide bonds present in the plasma resulting in formation of S-sulfonates. Number of rabbits used in the investigation prevented results from being considered statistically significant. However, no explanation was given for the use of rabbits.

Confidence index:

This study does not explicitly detail rationale behind choosing rabbits for the study, nor does it describe their gender. The age range was quite large, 4-22 months. No mention is made of whether rabbits were acclimatized or what pre-test conditions were. Feeding type was not specified, nor was information pertaining to the atmospheric conditions of the exposure device outlined. For the in vitro analysis, it is not clear how many samples were analyzed or the follow up length. Consequently, it would be difficult to reproduce the study.

Review – Confidence index ranking:

Low

Author: Bitron, M.D., Aharonson, E.F.	**Internal ID:** 224
Title: Delayed mortality of mice following inhalation of acute doses of CH_2O, SO_2, Cl_2 and Br_2	
Year:1978	
Abstract: Mortalities and times of death of mice were measured, during several weeks, after single respiratory exposures to air containing either of the 4 chemicals. The toxicity sequence observed, at 320 ppm, was Cl_2 greater than Br_2 greater than CH_2O greater than SO_2. For CH_2O and Br_2 at constant concentration, time of death depended markedly on exposure duration. The scant literature data enabled comparison of results for SO_2 only.	
Objective: No statement of objective.	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
1900ppm 1400ppm 900ppm	10-75 minutes 15-180 minutes 25-640 minutes	Mice	No info	1 month	male	16 per exposure per exposure duration	No information	unknown	900 ppm

Observations:

Both percent death during exposure and cumulated mortality increased with increasing exposure time (barring a single experiment at 1900 ppm in which a lone mouse out of 14 survived for 75 min). The dependence of the final cumulated mortality as a function of exposure time, was only significant after exposure to 1400 ppm. Two decidedly different aggregates of median time of death were obtained at each of the concentrations studied depending on whether or not deaths during exposure were excluded. For median time of death calculated excluding deaths during exposure, at 900 ppm the median time of death decrease notably with increasing exposure time. At each concentrations, both percentage of deaths during exposure and cumulated mortality increase with increasing exposure time. The major fraction of deaths occurred suddenly during exposure while any further mortality was delayed by at least 5 days. The toxicity sequence observed, at 320ppm, was $Cl_2>>Br_2>CH_2O>>SO_2$. For CH_2O and Fr_2 at constant concentration, time of death depended markedly on exposure duration. The scant literature data enabled comparison of results for SO_2 only.
*Statistically significant

Author's Conclusions:

The dose dependent mortality during exposure may suggest a mechanism of intoxication which differs from that causing delayed death. It is probably to be attributed to asphyxia following bronchostenosis and laryngeal spasm, as report for both humans and various mammals, including mice.

Review – Assessment:

A. Test animals:	(+)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+/-) c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (-)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate?

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C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water May be withheld optionally.*
D. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random Assignment) (-) Was a control group assayed? (N/A) Is the control regime appropriate?

	(+)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (-) Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (N/A) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results from this study indicated that percentage of deaths during exposure and cumulated mortality increased with increasing exposure time. Findings from this study might be applicable in occupational medicine and industrial hygiene, particularly to enhance the current bank of data on respiratory toxicities of common gases and vapors. However, the results are not toxicologically significant due to the extremely high SO_2 concentrations used: 1900,1400, and 900 ppm.

Confidence index:

While many confounding factors such as age and gender were accounted for in the materials and method section, no indication of pre-trial health was given. This study indicates that animals were in chambers during equilibration, but does not describe pre-testing conditions such as type and schedule of food. Furthermore, no explanation was given as to why particular dosages of SO_2 were used.

Furthermore, it is not clear whether the exposures were not randomly assigned and the control group only consisted of 2 mice of the 16.

There is no analysis for statistical significance, and only descriptive statistics were included. Unfortunately, the information provided on exposure conditions, procedures and data collection is too limited and the pretrial health of the mice is not discussed.

Review – Confidence index ranking:

Moderate

DRAFT

Author: Azoulay E. Soler P. Moreau J. Blayo MC	**Internal ID: 225**

Title: Effects of low-concentration NO_xSO_2 gas mixtures on lung structure and blood-oxygen affinity in rats.

Year: 1980

Abstract: The respiratory effects of low levels of SO_2 alone or associated with NO or NO_2 were studied in rats exposed for periods of one day to thirteen weeks. Control rats were exposed to ambient air. Both control and treated rats appeared similarly active and grew uniformly. Erythrocytic variables (hemoglobin, hematocrit, red cell counts, 2,3-DPG, glucose, lactate, methemoglobin) and oxyhemoglobin dissociation curves were determined at different times throughout exposure to the gaseous mixture. Blood variables of the exposed rats were not significantly different from those of controls, and hemoglobin affinity was not modified. Bronchiolar and tracheal epithelia were studied by scanning (TEM) to detect a possible loss of cilia. The alveolar walls were investigated by Light Microscopy and TEM after each period of exposure. No striking changes were observed in rat lung structures under Light Microscopy or TEM. Bronchiolar and tracheal epithelia were normally ciliated. No synergistic effects were produced by either SO_2 + NO or SO_2 + NO_2.

Objective: "Our purpose was to investigate the interaction between these (NO_x, SO_2) gases when mixed in roughly the same proportions as in the atmosphere. The experiment enabled use to determine the initial response of lung tissue to such pollutants after 24-72hours exposure."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
2.0ppm SO_2 and/or 2.0ppm NO_x	**Continuous 1, 3, 7 d 2, 4, 7 wk**	**Rat**	**Wistar**	**2mos (200g)**	**n/a**	**SO_2: N=8 x 6 SO_2 + NO_x: N=8 x 9**	**healthy**	**2.0ppm**	**n/a**

Observations:

Both control and treated rats appeared similarly active and grew uniformly. Erythrocytic variables and oxyhemoglobin dissociation curves were determined at difference times throughout exposure to the gaseous mixture. Blood variables of the exposed rats were not significantly different from those of controls, and hemoglobin affinity was not modified. Bronchiolar and tracheal epithelia were studied by scanning electron microscopy and transmission electron microscopy (TEM) to detect a possible loss of cilia. The alveolar walls were investigated by Light Microscopy and TEM after each period of exposure. No striking changes were observed in rat lung structures under Light Microscopy or TEM. Bronchiolar and tracheal epithelia were normally ciliated. No synergistic effects were produced by either SO_2 + NO or SO_2 + NO_2.

*Statistically significant

Author's Conclusions:

"In conclusion, it was of great physiological interest to prove that at gaseous concentrations within those of ambient air, rat behavior remains unaltered. The present study represents another new step in the air pollution field since previous authors generally investigated unusual pollution levels."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* +b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* ±c) Sex +d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
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C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* +✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* -✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.*

	-✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
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G. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? -✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective?

	+✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings suggest that low level exposures to either SO_2, SO_2+NO, or SO_2+NO_2 do not produce clinically significant respiratory effects, behavior in rats, blood variables and morphological features (lung histology), even after long periods of exposure (7 weeks). Furthermore, there is no evidence of synergistic effects between these compounds.

Confidence index:

Novel study looks at doses found in ambient air. Sufficient sample size for statistically significant results, even though none were obtained. Attempt to limit confounding variables by using same age and weight rats, fixative technique used for lung samples gave no structural artifacts, used pentotal as the anesthetic which did not induce histological alterations. Acclimation, feeding schedule and pre-test conditions were not mentioned. Exposure conditions and assessment methods were well defined – should be easily reproducible. Multiple parameters were used to make assessment. Could have tested multiple concentrations of SO_2.

Review – Confidence index ranking:

Moderate to high

Author: Amdur, M.O., Underhill, D.	**Internal ID:** 226
Title: The Effect of Various Aerosols on the Response of Guinea Pigs to Sulfur Dioxide	
Year:1968	
Abstract: Studies of the effects of various soluble and insoluble aerosols on the respiratory response of guinea pigs to sulfur dioxide are presented. The aerosols discussed are sodium, potassium, and manganous chlorides, ammonium thiocyanate, ferrous sulfate, sodium orthovanadate, activated and spectrographic carbon, manganese dioxide, iron oxide fume, open hearth dust, fly ash, and triphenyl phosphate. All these aerosols when given alone produced no aleteration in pulmonary flow resistance. From the point of view of air pollution toxicology, none would be classed as pulmonary irritants. Ferric sulfate, however, was irritant. In ability to potentiate the response to sulfur dioxide, sodium chloride, potassium chloride, and ammonium thiocyanate ranked in that order.	
Objective: To test the effect of molybdenum-exposed animals on sulfite oxidese and the subsequent susceptibility to bisulfite and sulfur dioxide toxicity.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
2ppm SO_2 2 ppm SO_2+ soluble aerosols(<10mg/m^3)	10 min	Guinea pigs	No info	No info	No info	16 n=10, 16, 15 for the three aerosols	No info	N/A	N/A
0.1-100 ppm SO_2 plus either iron oxide fume or manganese dioxide aerosols (8-10 mg/m$^{3)}$	No info					22 groups of animals ranging from n=6-n=40			
0.1-100 SO_2 alone and with six insoluble aerosols	No info					19 groups of animals ranging from n=6-n=23			
20 ppm SO_2 alone and with	1. 1 hour								

NaCl	2. 30 minutes								

Observations:

The aerosols alone did not produce a statistically significant change in pulmonary flow resistance. For the combined exposure, the potentiation ratio (response to gas aerosol per response to gas alone) was 2.4 for NaCl, 3.3 for KCl, and 5.0 for NH_4SCN. One isolated group of animals exposed to 10ppm sulfur dioxide and 8mg/m^3 activated carbon had a greater response than the group exposed to gas alone; this was statistically significant at $p<0.01$.
*Statistically significant

Author's Conclusions:

> "It is plain from data presented in this paper that all aerosols do not potentiate the response to sulfur dioxide. The experiments presented here indicate that the key to the role of sulfur dioxide in air pollution toxicology lies not in the gas itself but in its atmospheric chemistry."

Review – Assessment:

A. Test animals:	(+)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+/-)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source **Guideline: An established breeding facility or educational institution**

B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+/-)Was evidence provided to justify the dosing regime? (-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water* *May be withheld optionally.*
D. Equipment:	(-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined.

	Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water) (-)Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random Assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+/-)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined? (-)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (+)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective?

	(+)Were assumptions reasonable? (+)Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This paper examines effects of various aerosols on the response of guinea pigs to SO_2. The aerosols discussed are sodium, potassium, and manganous chlorides, ammonium thiocyanate, ferrous sulfate, sodium orthovanadate activated and spectrographic carbon, manganese dioxide, iron oxide fumes, open hearth dust, fly ash, and triphenyl phosphate. All these aerosols when given alone produced no alteration in pulmonary flow resistance. In ability to potentiate the response to sulfur dioxide, sodium chloride, potassium chloride, potassium chloride, and ammonium thiocyanate ranked in that order. The insoluble aerosols used were completely ineffective in potentiating the response to sulfur dioxide. The aerosols were all inert at the particle sizes and concentrations used, according to the author. The significance of the results is difficult to determine due to shortcomings in the study design.

Confidence index:

This paper makes mention of techniques to monitor flow-resistance but does not provide detail for the methods. No mention is made of pre-testing conditions Furthermore, it is unclear if animals were randomly assigned to exposure groups and the exposure durations of the different experiments are not well defined. Consequently, the study would be difficult to reproduce. Data in the study expands on previous work done by the author and similar trends are observed in terms of the potentiation effect of aerosol and prolonged effect on resistance. However, only a single endpoint was used to assess effects – perhaps other criteria should have also been measured.

Review – Confidence index ranking:

Low

Author: Amdur MO. Underhill DW.

Internal ID: 227

Title: Response of guinea pigs to a combination of sulfur dioxide and open hearth dust.

Year: 1970

Abstract: Measurement of alterations in the mechanical behavior of the lungs of guinea pigs may be used to assess the irritant potency of air pollutants. By examining the response to an irritant gas alone and in the presence of a known amount of particulate material it is possible to determine whether or not the particulate material has caused a potentiation of the response. This technique has been used to examine the effect of redispersed open-hearth dust on the response to sulfur dioxide. The open-hearth dust used was material obtained from electrostatic precipitators. The system used for re-dispersal of this material will be described. The open-hearth dust alone produced no detectable respiratory response even when it used at a concentration as high as 7mg/m^3. It was also without effect on the response to several levels of sulfur dioxide. The findings were similar for an aerosol of iron oxide. This is in contrast to soluble iron salts, which do potentiate the response to sulfur dioxide. These contrasting findings suggest that the potentiation of sulfur dioxide is mediated by solubility in a droplet followed by catalytic oxidation to sulfuric acid.

Objective: "The effect of iron oxide fume and redispersed open hearth dust on the respiration of guinea pigs was measured and the response to sulfur dioxide alone was compared with the response to the gas in the presence of iron oxide fume and open hearth dust."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
1.5-26ppm SO_2 and/or 1.0-24.0mg/m^3 Fe_2O_3	**1 or 2 hrs**	**Guinea pig**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**n/a**	**0.16ppm**

Observations:

Exposure to levels of SO_2 alone, ranging from 1.5-26ppm demonstrated significantly increased levels of airway resistance relative to controls.

Exposure to SO_2 and Fe_2O_3 demonstrated increases relative to unexposed controls at all levels (though not different from the effects of SO_2 alone) except 21ppm SO_2 and 4.8mg/m^3 Fe_2O_3, a finding which cannot be easily explained. No statistically significant differences in airway resistance between SO_2 and SO_2+open hearth dust were noted.

*Statistically significant

Author's Conclusions:

"The response of guinea pigs to iron oxide fume and redispersed open hearth dust was studied. Neither of these substances produced any detectable respiratory response in the animals exposed to levels of 7 to 20mg/m^3. Neither iron oxide nor open hearth dust affected the response to sulfur dioxide. The changes observed on exposure to such combinations could be accounted for on the basis of the response to the gas alone at corresponding concentrations."

Review – Assessment:

A. Test animals:	±✎ Is the number of animals sufficient to produce statistically significant results? □ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie □ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age □ *Guideline: Adult, i.e.,8-12 weeks.* ±c) Sex ±d) Pre-test health □ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation □ *Guideline: Below 20% variation* ±f) Source □ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? □ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? +/-✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? □ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? ±✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? □ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? □ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? □ *Guideline: Animals may be grouped by sex, or individually.*

	-✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +/-✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? ±✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? ±✎ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +/-✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +/-✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? -✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective?

	+/-✓ Were assumptions reasonable? +✓ Are the methods reproducible? +✓ Did either the study design or the statistical analysis limit confounding variables? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings suggest that airway responsiveness to SO_2 is not potentiated by either iron oxide fume or open-hearth dust. The lack of synergism implies that concurrent exposures to SO_2 and these compounds are not more harmful than SO2 alone, and may have implications for understanding the mechanism by which these compounds affect the airway.

Confidence index:

Study is not novel, previous study had shown that potentiation of SO_2 is mediated by solubility and catalytic oxidation to H_2SO_4, this mechanism is further reinforced by these data. Sufficient sample size to generate statistically significant results. Unknown age, weight or sex of animals would make pooled results from the animals difficult to interpret if any of these factors were significantly different, despite their use of each animal as its own control. Raw data not provided to assess these variables. Significance was assigned to most of the data, no mention of method used for statistical analysis. Also unknown if animals were re-exposed multiple times. Exposure conditions and assessment were not well defined – study would not be easily reproducible. Only a single endpoint of pulmonary flow resistance was assessed – multiple parameters should have been looked at.

Review – Confidence index ranking:

Low to Moderate

Author: Amdur MO. McCarthy JF. Gill MW.	**Internal ID: 229**

Title: Effect of mixing conditions on irritant potency of zinc oxide and sulfur dioxide.

Year: 1983

Abstract: Measurement of mechanics of respiration in guinea pigs was used to assess the irritant potency of zinc oxide and sulfur dioxide mixed under different conditions of temperature and humidity. Concentrations were 1-2 mg/m3 zinc oxide and 1 ppm sulfur dioxide. Dry conditions of mixing (Chamber RH 30%) either at 24 degrees C in the exposure chamber or at 480 degrees C in a dry furnace gave a biological response which could be completely accounted for by responses to zinc oxide and/or sulfur dioxide alone. Chemical examination of the aerosols did not indicate the formation of particulate sulfur species. Zinc oxide and sulfur dioxide mixed dry at 480 degrees C and fed into the exposure chamber at 80% RH reacted to produce an irritant aerosol as evidenced by a rapid increase to levels 29% above control; reversal was rapid when exposure ended. Chemical studies indicated the presence of sulfite on these aerosols. Addition of water vapor to the furnace during mixing at 480 degrees C produced a different irritant aerosol. The resistance rose slowly to 19% above control values and remained elevated during the post-exposure hour. Chemical studies indicated the presence of sulfate, sulfite, and adsorbed sulfur trioxide on these aerosols.

Objective: "This study was designed to examine the effect of conditions of mixing on the irritant potency of combinations of zinc oxide and sulfur dioxide."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
~1ppm SO_2 and/or ~2mg/m^3 ZnO	**1 hour**	**Guinea pig**	**Hartley**	**n/a**	**male**	**20**	**n/a**	**n/a**	**1ppm**

Observations:

Effects of:
1) 1ppm SO_2 alone: resistance increases 11%, compliance decreases 12% ($p<0.05$ for each)
2) ZnO + SO_2, mixed @ 24°C and 30%RH: non-significant 8% increase in resistance, 12% decrease in compliance ($p<0.01$), 6% decrease in minute volume (-6%, $p<0.05$)
3) ZnO + $_{SO2,}$ mixed @ 480°C and 30%RH: 12% increase in resistance, 12% decrease in compliance ($P<0.05$ for each), 6% decrease in minute volume ($p<0.05$)
4) control – 80% RH: no significant changes
5) ZnO, 80% RH: no significant changes
6) ZnO + SO_2, mixed @ 480°C and 80%RH: 29% increase in resistance ($p<0.01$), compliance decreased by 13% ($p<0.001$)
7) ZnO + SO_2, mixed humid @ 480°C and 30%RH: 19% increase in resistance ($p<0.01$), compliance 8% non-significant decrease

*Statistically significant

Author's Conclusions:

"It is evident that there are qualitative differences in the sulfur species formed under these conditions of mixing. The present analytical results are consistent with physiochemical data obtained by others. The response obtained in animal exposures to these two conditions of mixing was indicative of the formation of an in irritant aerosol. The exact nature of the response differed which is in agreement with the analytical observation of different sulfur species."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex -d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* +f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate?

	☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* +✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* +✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? ±✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported?

	+✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective? +✏ Were assumptions reasonable? +✏ Are the methods reproducible? +✏ Did either the study design or the statistical analysis limit confounding variables? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The authors demonstrated that not only the chemical composition of compounds, but the manner in which they are formed, can have implications for the type of respiratory affects they will manifest when inhaled. This research has implications for the manner in which future exposure studies are done and also for persons exposed in industry where extreme conditions are present (e.g., metal furnace workers), so that the compounds the persons are exposed to there may differ considerably from those studied under laboratory circumstances, and may have different effects. This study points out the need to examine physical aspects of air pollutant formation, as well as chemical, in order to fully understand their human health effects.

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Confidence index:

Sample size appears to be sufficient for all treatments, and a good variety of treatments were used to examine potential respiratory effects. Age of guinea pigs was not mentioned, nor were pre-experimental conditions. The number of animals grouped into test groups was not mentioned, nor was any period of acclimation. No mention was made of whether animals were randomly assigned to groups, and if so, how the randomization process was done. Exposure conditions were not well defined, but assessment methods were well defined. Attempt to limit confounding factors by using same sex animals and having each animal serve as its own internal control.

Review – Confidence index ranking:

Moderate

Author: Abraham, W.M., Oliver, W., Welker, M.J., King, M.M., Wanner, A., Sackner, M.A.

Internal ID: 230

Title: Differences in airway reactivity in normal and allergic sheep after exposure to sulfur dioxide

Year: 1981

Abstract: The effect of breathing 5 ppm sulfur dioxide (SO_2) on airway reactivity was studied in both normal and allergic conscious sheep. Allergic sheep were defined as animals in which inhalation of *Ascaris suum* extract resulted in bronchospasm as evidenced by an increase in mean pulmonary flow resistance (R_L), hyperinflation, and a fall in dynamic compliance. Airway reactivity was assessed by measuring the increase of R_L after 18 breaths of 0.25% carbachol (c), from an initial R_L value obtained after 18 breaths of buffered saline (s) [R_L(c-s)]. R_L and R_L(c-s) were determined prior to, immediately after, and 24 h after exposure to 5 ppm SO_2 for 4 h. In both groups R_L remained unchanged after SO_2 exposure. Prior to exposure, R_L(c-s) was not significantly different in seven normal (0.3 +/- 0.1) and seven allergic sheep [0.4 +/- 0.2 (SD) cmH_2O X l-1 X s], and there was no significant change in R_L (c-s) immediately after SO_2 exposure in either group. Twenty-four h later, R_L(c-s) R_L(c-s) increased to 0.7 +/- 0.8 (P less than 0.2) in normal and to 1.8 +/- 0.9 cmH_2O X l-1 X s (P less than 0.01) in allergic sheep. Because the increase in R_L(c-s) after 24 h was greater (P less than 0.01) in allergic than in normal sheep, we conclude that SO_2 exposure increased airway reactivity more in the former than in the latter.

Objective: To use the measurement of airway reactivity for the purpose of characterizing the airway effects produced by 5ppm SO_2 in two distinct groups of conscious sheep: normal sheep and sheep previously sensitized to *Ascaris suum* antigen (allergic sheep).

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
5 ppm SO_2	4 hours	sheep	No info	No info	No info	7 normal and 7 allergic sheep	No info	N/A	N/A

Observations:

Exposure to 5 ppm SO_2 in Normal and Allergic Sheep

Within group changes in pulmonary mechanics before and after SO_2 exposure

SO_2 exposure did not significantly alter R_L (mean pulmonary flow resistance), V_T(tidal volume), f(breathing frequency), Cdyn (dynamic lung compliance), or Cst(static lung compliance) at any time in either the normal or allergic sheep, compared with the respective preexposure values. Twenty-four hours after SO_2 exposure, R_L(c-s)(see abstract) increased significantly in the allergic sheep. This 24-hour increase in R_L(c-s) was also significantly different from the immediate postexposure increase for the allergic sheep. There were no significant within-group changes due to carbachol inhalation prior to or following SO_2 exposure in V_T, f, Cdyn, or Cst.

Differences in response to carbachol inhalation before and after SO_2 exposure between normal and allergic sheep Between group comparisons of the absolute changes n pulmonary mechanics due to carbachol inhalation before and after SO_2 exposure indicated that there were significant differences in R_L(c-s) and V_T(c-s) between groups. A planned comparison test indicated that R_L(c-s) between groups was significantly different ($p<0.01$) 24 h after exposure. The only other significant difference between groups occurred before SO_2 exposure, when the change in V_T after carbachol inhalation was significantly greater in the allergic than the normal group.
*Statistically significant

Author's Conclusions:

This study suggests that a short-term exposure to 5 ppm SO_2 1) does not produce significant changes in airway function in normal and allergic sheep, 2) does not increase airway reactivity to aerosolized carbachol in normal sheep, but 3) does produce a significant changes in airway reactivity in allergic sheep.

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+/-)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective?

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C. Housing/Feeding:	(N/A) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (N/A) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (N/A) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water May be withheld optionally.*
D. Equipment:	(-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease

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F. Data collection:	(+)Were appropriate endpoints examined? (-)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (+)Are the results novel? (+/-)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+/-)Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially

	bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study tried to assess the effects of SO_2 exposure on airway reactivity in normal and allergic sheep.

In both normal and allergic sheep, the R_L remains unchanged after SO_2 exposure. There was no significant change in R_L(c-s) immediately after SO_2 exposure. In either group. Twenty-four hours post-exposure, the R_L(c-s) increased significantly in both groups. The SO_2 concentration was relatively low, and may have been too low to elicit a response. Also, the peak response could have taken place between the immediate and 24-hour measurement periods. Future studies might attempt to extrapolate findings to humans, to determine if SO_2 exposure increased airway reactivity more in normal than allergic subjects.

Confidence index:

Details such as feeding and watering regiment, including environmental conditions of the test chamber were not mentioned, nor was acclimation period. In addition, possible confounding factors such as gender, and age were not mentioned. Furthermore, no information was given as to how animals were divided into groups. Justification was given for the use of sheep (allergic sheep have antigen induced airway responses similar to those that occur in patients with allergic airway disease). However, the justification includes reference to only one study. In addition, nine sheep were classified as allergic, but only 7 were used. The reasons for exclusion are not given.

Some of the animals used in the challenge were also used in the SO_2 exposure. Some animals took part in the challenge before the SO_2 exposure experiments and some animals took part after the SO_2 exposure experiments. This may have affected the responses.

Review – Confidence index ranking:

Moderate

Author: Abraham WM. Oliver W Jr. Welker MJ. King M. Chapman GA. Yerger L. Maurer DR. Sielczak M. Wanner A. Sackner MA.

Internal ID: 231

Title: Sulfur dioxide induced airway hyperreactivity in allergic sheep.

Year: 1980

Abstract: The effects of a four-hour exposure (via a Plexiglas hood) to sulfur dioxide (SO_2) on airway reactivity was studied in both normal and allergic conscious sheep. Allergic sheep were defined as animals in whom inhalation of Ascaris suum extract resulted in an increase in mean pulmonary flow resistance (RL). Airway reactivity (delta RL) was assessed by measuring the increase in RL after 18 breaths of 0.25% carbachol, from an initial value obtained after 18 breaths of buffered saline. RL and delta RL were determined prior to, immediately after and 24 hours following SO_2 exposure in three groups of sheep: six normal sheep exposed to 5 ppm SO_2 (group A); six normal sheep exposed to 10 ppm SO_2 (group B) and seven allergic sheep exposed to 5 ppm SO_2 (group C). RL was not affected by SO_2 exposure in any group but both groups B and C showed increases in delta RL 24 hours after exposure. Since the increase in delta RL was greater in group C than in either groups A or B, we conclude that allergic sheep have enhanced susceptibility to the injurious airway effects of SO_2.

Objective: "In the present study we have attempted to gain some insights into the possibility of increased airway sensitivities to SO_2 in different populations by examining the airway reactivity to aerosolized carbachol in two distinct groups of conscious sheep: normal sheep and sheep previously sensitized to Ascaris suum antigen (that is, allergic sheep)."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
5-10ppm	4hrs	Sheep	n/a	n/a 27-48kg	n/a	27	Healthy & Allergic	n/a	5ppm

Observations:

In six normal sheep, exposure to 5 ppm SO_2 did not significantly alter resistance or airway reactivity. Static lung compliance was also unchanged. In six normal sheep exposed to 1) ppm SO_2 resistance was again unchanged but exposure did result in a significant increase in airway reactivity 24 hours later.* Although exposure to 10 ppm SO_2 did increase reactivity in normal sheep, this was significantly less than the increase observed in the seven allergic sheep*.

*Statistically significant

Author's Conclusions:

This study suggests that the airways of allergic sheep are sensitive to levels of SO_2 that do not affect normal sheep, thus possibly reflecting an inherent difference in the initial state of the airways for the two populations. These results are consistent with previous studies in man that suggested that the airways of individuals with abnormal lung function may be sensitive to levels of gaseous pollutants that do not readily affect normal individuals.

Review – Assessment:

A. Test animals:	+✏ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✏ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex +d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✏ Were the chosen exposure levels appropriate to investigate the primary objective? -✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ±✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? ±✏ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? -✏ Was evidence provided to justify the dosing regime? -✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✏ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✏ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* -✏ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.*

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G. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective?

	+✓ Were assumptions reasonable? +✓ Are the methods reproducible? -✓ Did either the study design or the statistical analysis limit confounding variables? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study suggests that the airways of allergic sheep are sensitive to levels of SO_2 that do not affect normal sheep, thus possibly reflecting an inherent difference in the initial state of the airways for the two populations. These results are consistent with previous studies in man that suggested that the airways of individuals with abnormal lung function may be sensitive to levels of gaseous pollutants that do not readily affect normal individuals. They also presented data showing that the allergic sheep required a much higher level of carbachol (to distinguish them from normal sheep) than is usually necessary to identify asthmatic patients – mechanism of increased airway activity may not be the same.

Confidence index:

The methods of exposure seem appropriate, though the methods of gas-generation and animal characteristics are explained in very poor detail. Details such as feeding and watering regiment were not mentioned nor were environmental conditions of the test chamber. Assessment methods were well defined – study should be easily reproducible. Only used resistance to assess airway reactivity, only a single challenge (carbachol) used – should have assessed multiple parameters to see effect on lung mechanics and also tried additional challenges. Unknown age, large weight differences and unknown sex of animals used. Sample size, however, is sufficient (n>=6 for all treatments). Follow-up was also only for 24 hours but the lack of a more thorough follow-up, is unlikely to affect the conclusions made in this study. Extrapolation not warranted since mechanism for increased hyper reactivity is not known, either in sheep or humans.

Review – Confidence index ranking:

Moderate

Author: Callanan D. Dixon M. Widdicombe JG. Wise JC.	**Internal ID: 233**
Title: Responses of geese to inhalation of irritant gases and injections of phenyl diguanide.	
Year: 1974	
Abstract: Changes in blood pressure, heart rate, tidal volume, breathing frequency, laryngeal airflow resistance and lower airway resistance have been measured in anesthetized geese. Inhalation of 100-400ppm sulfur dioxide caused hypertension, tachycardia, apnea followed by rapid shallow breathing and a decrease in lower airway (bronchial) resistance. The responses persisted after cutting both vagus nerves in the chest. The afferent mechanisms are discussed. Insufflation of 1-100ppm ammonia vapor into the upper trachea and larynx caused hypertension, tachycardia, apnea, and slowing of breathing. Insufflation of 5-8% carbon dioxide into the larynx caused no changes in any of the variables measured. Intravenous injections of phenyl diguanide caused hypotension, bradycardia, apnea, and slowing of breathing and laryngeal constriction by a vagal reflex, presumably from lung nociceptive endings.	
Objective: "We have investigated the actions of inhaled sulfur dioxide on breathing, the cardiovascular system and airway resistances in geese. We also describe their responses to carbon dioxide and ammonia in the cranial trachea and larynx and to intravenous injections of phenyl diguanide, a drug which stimulates alveolar nociceptive endings in mammals."	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
100-400ppm	**1-3min (until response observed, or 3 min if no response)**	**Hybrid goose**	***Anser anser / Cygnopsis cygnoid***	**Adult (5-6kg)**	**n/a**	**32**	**n/a**		

Observations:

After administration of SO_2: "Blood pressure and heart rate were consistently increased. Tidal volume changes were small and rather inconsistent. Tachypnea was nearly always seen, preceded by apnea (for 5-20sec) in about half the tests. Upper airway (laryngeal) resistance increased briefly in two of seven tests. Lower airway resistance was decreased in nine of 15 experiments. The control value (mean±SEM) was 8.7±1.1cm H_2O/l s and the mean change for all tests was -15.8±4.70% ($p<0.01$), and that for the tests which showed a decrease in resistance was -29.0±2.61% ($p<0.01$). When SO_2 was given after bilateral vagotomy or during cold-block of the vagi, the results were qualitatively similar to those with intact vagal conduction. Vagotomy itself seemed to increase lower airway resistance (control value, 11,9±0.93cm H_20/l s), but the proportionate changes in resistance caused by SO_2 were very similar to those given above for tests with vagal conduction blocked."
*Statistically significant

Author's Conclusions:

"There are striking differences between the responses of the goose compared with those of mammals on inhalation of chemical irritants through a tracheal

catheter… Perhaps the most unexpected difference is the decrease in lower airway resistance seen in the goose… the site of this bronchodilation has not been confirmed."

"Our results support and extend the evidence for reflexes from the respiratory tract of birds which are elicited by inhalation of irritant gases. A number of clear differences between the avian and mammalian reflexes have been established; this applies not only to the afferent systems of the reflexes, but also to the motor responses. In particular the reflex control of the avian airway caliber, both of the larynx and of the intrathoracic airways, exhibits large differences from that of mammals. Further studies on the nervous control of airways in the bird are needed to clarify the mechanisms involved."

Review – Assessment:

A. Test animals:	+✏ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✏ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* +b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* ±c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
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G. Data analysis:	±✏ Are the statistical methods used appropriate? ±✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +/-✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective? +✏ Were assumptions reasonable? +✏ Are the methods reproducible? +✏ Did either the study design or the statistical analysis limit confounding variables? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study identifies that there are some striking differences between geese and other mammals following inhalation of SO_2 through a tracheal catheter. This applies not only to the afferent systems of the reflexes, but also the motor responses. In particular the reflex control of avian airway caliber, both of the larynx and of the intrathoracic airways, exhibits large differences from that of mammals. Further studies on the nervous control of airways in the bird are needed to clarify the mechanisms involved.

Confidence index:

The methods of the study are explained well, and treatments studying SO_2 effects have adequate sample size (n>=5). The generation of gas is not described explicitly, but samples were taken during the experiment to confirm the correct concentration. While exposure to SO_2 varied from 1-3 minutes, this is not expected to be a major problem, so that while it may affect the quantitative results observed, the qualitative results should be by and large unaffected. Each goose served as its own internal control, also tested air controls with the caudal tracheal catheter used for SO_2 inhalation and found no change in response to any of the variables tested – tried to limit confounding factors. Study looked at multiple parameters to assess the effect of SO_2 – breathing, cardiovascular system and airway resistance. Discussed possible mechanisms for differences in response for geese versus other mammals. Data are in agreement with other studies done in other avian species. Exposure and assessment methods are well defined and should be easily reproducible with the exception that the range of exposure is not well defined (100-400 ppm).

Review – Confidence index ranking:

Moderate to high

Author: Davies, A., Dixon, M., Callanan, D., Huszczuk, A., Widdicombe, J.G., Wise, J.C.M.	**Internal ID:** 234

Title: Lung Reflexes in Rabbits During Pulmonary Stretch Receptor Block By Sulphur Dioxide

Year: 1978

Abstract: Anaesthetized rabbits were given 200 ppm sulphur dioxide to breathe for 10 min. This abolished activity in 23 of 26 pulmonary stretch receptors, while leaving that of lung irritant receptors unimpaired. The Breuer-Hering reflex was abolished and breathing became deeper and slower. Inspiratory time (tI) was increased and expiratory time (tE) decreased. Subsequent vagotomy increased tidal volume (VT), tI and tE. In animals with stretch receptors blocked, injections of phenyl diguanide and histamine still increased breathing frequency and decreased VT, indicating that reflexes from lung irritant and J-receptors were intact. Inhalation of 8% CO_2 caused a bigger increase in frequency and tidal volume in rabbits with stretch receptor block compared with controls or those after vagotomy. Induction of pneumothorax with stretch receptor block transiently prolonged tI and shortened tE; removal of the pneumothorax also transiently shortened tE and usually also decreased tI. The results suggest that lung irritant receptors reflexly shorten tE in all our experimental conditions, but have various effects on tI which may depend on the timing of the irritant receptor discharge and refractoriness of the inspiratory response.

Objective: To determine lung reflexes in rabbits during pulmonary stretch receptor block by sulphur dioxide.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
200 ppm SO_2	10 min periods (1-5 periods)	Rabbit	New Zealand	No info	No info	Varied for each experiment N=6 to N=25	No info	N/A	N/A

Observations:

Pattern of Breathing

By the end of the 10 min exposure, total lung resistance was only slightly higher than the control value. Compliance was not significantly changed by exposure to SO_2. After removal of SO_2, the pattern of breathing returned to control values in about 20-60 minutes. In 5 out of 5 rabbits after vagotomy, administration of SO_2 had no significant effects on pattern of breathing or on phrenic discharge. Vagotomy, like exposure to SO_2, significantly increased the duration and maximum height of the phrenic integral.

Receptor Activity

To analyze the cause of the change in pattern of breathing induced by SO_2, the single-fibre activity from pulmonary stretch and lung irritant receptors in bilaterally vagotomized rabbits was recorded. Twenty three of the twenty six slowly adapting pulmonary stretch receptors studied in paralyzed ventilated rabbits were completely blocked by 1-8 minutes exposure to 200ppm SO_2 .All the pulmonary stretch receptors recovered their spontaneous activity, quantitatively to control values, within 20-60 minutes of removal of SO_2. There were no significant changes in the activity of irritant receptors.

Breuer-Hering inflation reflex

Since SO_2 greatly diminished the activity of pulmonary stretch receptors, which mediate the Breuer-Hering reflex, the strength of the reflex before and after SO_2 exposure was tested before and after SO_2 exposure. There were no statistically significant findings. In four of ten rabbits the reflex was completely abolished after SO_2 exposure. At the end of 60 min after removal of the SO_2 the size of the Breuer Hering reflex had been restored to its control value.

Histamine

Exposure to histamine produced a significant decrease in t_E and V_T in rabbits with stretch receptor activity intact.

Phenyl diguanide

The effect of phenyl diguanide on end-expiratory volume was measured in six rabbits. Before stretch receptor block it increased significantly ($p<0.05$). After the block, statistically significant increases occurred in all animals.

Histology

No damage to the lungs, bronchi or trachea in any of the five animals exposed to SO_2 (for 1-5 periods of 10 min during 4-5 hours) and studied histologically.

Blood Gases and pH

The only significant change after exposure to SO_2 was the decrease in P_{CO2} although this was not reflected in end-tidal CO_2 values in a different group of rabbits.

*Statistically significant

Author's Conclusions:

The results suggest that lung irritant receptors reflexly shorten t_E in all our experimental conditions, but have various effects on t_I which may depend on the timing of the irritant receptor discharge and refractoriness of the inspiratory response.

Review – Assessment:

A. Test animals:	(+)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+/-)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source **Guideline: An established breeding facility or educational institution**
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	Guideline: Longer than 14 days.
F. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+/-) Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (+)Are the results novel? (+/-)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (N/A) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study used SO_2 to block pulmonary stretch receptors in rabbits (this effect of SO_2 was previously characterized) and then tested lung irritant and J-reflexes in response to various stimuli. Results indicated that SO_2 did not block lung irritant or J-receptors but could not identify whether SO_2 caused stimulation or sensitization of these receptors. No extrapolations made to whether this could be used as a model to characterize human responses. While results conflict with a previous study that indicated that J-receptors shorten tI in the rabbit, it is unclear whether J-receptors may have also contributed to responses ascribed to irritant responses in this study.

Confidence index:

This study accounted for factors such as number of animals and strain of species, but did not include information on gender, age, or previous health of animals involved. However, the paper references another paper for more details on methodology. Information such as pre-exposure conditions were not reported, including climatic conditions and whether rabbits were acclimatized prior to the experiment. It was also not clear how animals were divided into subject groups and whether randomization was used to divide animals. There was no clear information on a control regime. Furthermore, the information provided for the test animals was too limited. More information is needed on the mode of administration (gas mixture was dragged across the trachea) and there was no discussion on whether the anesthetic may affect lung function. The exposure duration was not well defined. Finally, the timing and frequency of assessment was not clear and the period of observation was inadequate.

Review – Confidence index ranking:

Low to moderate

Author: Ferin J. Leach LJ.	**Internal ID: 235**

Title: The effect of SO_2 on lung clearance of TiO_2 particles in rats.

Year: 1973

Abstract: By use of the nontoxic-particle-challenge system, the integrated alveolar clearance was measured in rats by serially sacrificing them after exposure to TiO_2 aerosol and determining the amount of TiO_2 retained in the lung. Prior to exposure to TiO_2, groups of animals were exposed to 0.1, 1, or 20ppm of SO_2 for 7hours/day, 5 days/week for a total exposure of 70-170 hours. The results indicated that SO_2 affects the clearance of "inert" particles. The lowest exposures showed slight stimulation or no effect on clearance, whereas a depressing of clearance was observed after 170hours of exposure at 1ppm. Short term exposure at higher concentrations appear to be tolerated better than longer exposures at low concentrations.

Objective: "In order to assess the effects of SO_2 we decided to use a different lung function test, namely particle clearance. Hopefully this approach would provide another method and new information on the combined effect of SO2 and airborne particles. Further, rats were exposed to low concentrations of SO_2 repeatedly for seven hours per day."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0.1, 1, 20ppm	**7h/d 5d/wk for 70-170hrs total**	**Rat**	**Long-Evans**	**n/a ~200g**	**Male**	**420**	**n/a**	**n/a**	**0.1ppm**

Observations:

At 0.1ppm: "In two experiments we found a slight but statistically significant increase in clearance."($p<0.02$)
At 1.0ppm: "With ten to twenty exposures, the clearance was at control levels or better. However, exposed to this concentration for 25days, rats showed a depressed clearance of particles, as measured 25days after TiO_2 inhalation." ($p<0.01$)
At 20ppm: "The results indicated a slight decrease of clearance when compared to control values." ($p<0.05$)

*Statistically significant

Author's Conclusions:

"The results indicate that SO_2 exposure affect the clearance of "inert" particles. After a slight stimulation (70hoursat 0.1ppm) no effect, as well as a depressing of TiO_2 clearance (170hours at 1ppm) was observed. It seems that short term higher concentrations (20ppm) are tolerated better than longer lasting lower exposures."
"In our judgement the results obtained showed that the lung clearance technique is a sensitive and meaningful indicator of adverse air pollutant levels."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* +✎ Are animal characteristics adequately recorded and appropriate for the objective of the study?

	+a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	±✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ±✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? ±✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water*

	may be withheld optionally.
D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? +✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? ±✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? ±✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable?

	+✎ Is the paper in a peer-reviewed journal?
	+✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The study demonstrates that even low-level SO_2 exposures can impair lung clearance in exposed rats, and that long-term lower level exposures have greater effects than shorter, higher level ones. These findings could have implications for policy and regulation of exposures if similar patterns are seen in humans, as even low levels of SO2 pollution can potentially negatively affect lung clearance. No simple dose response was seen, some increased particle clearance, while some decreased particle clearance. No extrapolations made as to whether the model could be used to characterize human responses. Method could not be used because it involves excising lung to measure particle content remaining.

Confidence index:

This study uses a new method to evaluate lung clearance in response to SO_2 inhalation. While this method has not been proven, it appears to be a valid method of measuring lung clearance. Sample size is large (n=420), a gradient of exposures was used, and all results are statistically analyzed. No dose response was seen, some were beneficial and some were detrimental – did not use standard methods of measuring respiratory function to validate the data. Did not use conventional means to interpret exposures as a product of time and concentration, instead used product of time to the fourth power and concentration to create an exposure index – made the data fit an arbitrarily defined equation.

Review – Confidence index ranking:

Moderate

Author: Etlik, O., Tomur,A., Kutman, M.N., Yorukan, S., Duman, O. **Internal ID:** 236

Title: The Effects of Sulfur Dioxide Inhalation and Antioxidant Vitamins on Red Blood Cell Lipoperoxidation

Year: 1995

Abstract: An attempt was made to determine whether sulfur dioxide (SO_2,) inhalation at 10 ppm, 1 hr daily, for 30 days induces oxidant stress and whether vitamin E (40 mg/kg) together with vitamin C (200 mg/kg), administered intraperitoneally once in every 3 days, can reduce the damage in red blood cell membranes of guinea pigs. Malonyldialdehyde (MDA) levels, osmotic fragility ratios, and methemoglobin and sulfhemoglobin values were significantly higher in the SO_2-treated group compared with the control group ($P < 0.05$), and marked decreases in MDA levels and osmotic fragility ratios were determined in the group treated with SO_2 + antioxidant vitamins ($P < 0.05$)

Objective: To investigate the effects of SO_2 inhalation on lipoperoxidation and osmotic fragility of RBC membranes and to see if there is any protective effect of antioxidant vitamins.

Overall study design:

Exposure level	Exposure frequency/ duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
10 ppm (Groups B and C were exposed to SO_2 and Group A was the control)	1hr/d, 30d	Guinea pigs	No info	No info	No info	36 (3 groups of 12)	No info	N/A	N/A

Observations:

Methemoglobin and Sulfhemoglobinemia

SO_2 inhalation resulted in higher ratios in groups B and C ($p<0.05$).

Lipoperoxidation

MDA (see abstract) levels were significantly higher in group B than in the control group and a marked decrease due to vitamin administration was observed in group C compared with group B ($p<0.05$).

Osmotic Fragility

Osmotic fragility was significantly higher in group B than in group A ($p<0.05$). The values of group C were significantly lower compared with those of group B ($p<0.05$).

*Statistically significant

Author's Conclusions:

In the present study, decreases in lipoperoxidiaton and osmotic fragility ratios were determined in group C; therefore, antioxidant vitamin combinations may be useful in cardiac patents and elderly people, especially during periods of heavy pollution.

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+/-)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+/-)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source **Guideline: An established breeding facility or educational institution**
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the

	equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (+) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (+) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water May be withheld optionally.*
D. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+) Were test animals randomly assigned to exposure groups? (+) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random Assignment)

	(+) Was a control group assayed? (+) Is the control regime appropriate? (+/-)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined? (-)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (+) Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

In the present study, SO_2 inhalation caused significant increases in methemoglobin and sulfhemoglobin ratios, MDA levels, and osmotic fragility ratios. Higher meth- and sulfhemoglobin values due to SO_2 inhalation were also determined in group C compared with group A($p<0.01$). However, MDA levels and osmotic fragility rations were significantly lower in group C than in group B. From these studies, the findings indicate that the combination of vitamins E and C may reduce lipoperoxidation and oxidant damage. Clinically, anti-oxidant vitamin combinations might be useful in cardiac patients and the elderly, especially during periods of high pollution.

Confidence index:

Gender and species of guinea pig, along with pre-exposure health, were not described. Furthermore, no gradient of exposure levels was mentioned. However, the author does describe how animals were divided into three groups and type and amount of feed were specified. There is no information on the sex, age or pretrial health of the animals. However, assessment was only performed once at the end of the study and more information is needed on blood analysis. Despite this, exposure conditions and experimental assessments were well defined – study should be easily reproducible and methods would easily transfer to testing in humans.

Review – Confidence index ranking:

Moderate

Author: Balchum OJ, Dybicki J, Meneely GR	**Internal ID: 237**
Title: Pulmonary resistance and compliance with concurrent radioactive sulfur distribution in dogs breathing $S^{35}O_2$.	
Year: 1960	
Abstract: Ten dogs breathed $S^{35}O_2$ via a tracheal cannula in concentrations ranging from 1.8 to 148ppm. There was a decrease in the compliance of the lungs and thorax after 10-15min inhalation, and an immediate and marked increase in the nonelastic resistance to breathing. S^{35} was found to be highest in concentration in the trachea, lungs, and hilar lymph nodes, and next highest in the kidneys and esophagus. A relatively uniform and low concentration was noted in other tissues, such as the heart muscle, liver, spleen, and striated muscle. $S^{35}O_2$ when inhaled was absorbed quickly, distributed widely to many tissues, and readily gained access to many tissues, including the brain.	
Objective: "We have measured, concurrently, a) the effect of SO_2 on pulmonary function and b) its concentration and localization in the blood and tissues. The following report describes the results of these experiments in 10 dogs breathing measured volumes of $S^{35}O_2$ of known concentration and activity via a tracheostomy for periods of from 30 to 40minutes."	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
1.8-148ppm	**30-40min**	**dogs**	**n/a**	**n/a**	**n/a**	**10**	**n/a**		

Observations:

Compliance of lungs and thorax in dogs exposed to 1.8-148ppm SO_2 for 30-40min, on average by 8.5% ($p<0.05$).
Resistance increased by 150-300% during exposure, but decreased to control levels shortly after.
Marked S^{35} was found in a number of tissues, but with most concentrated in the trachea and bronchi. Concentration of S^{35} decreased from the upper to the lower trachea, with concentration in the lungs 20% of that in the trachea.

*Statistically significant

Author's Conclusions:

"There was a significant decrease in the compliance of the thorax and lungs in all but one dog, and an increase in non-elastic resistance to breathing in all animals soon after the onset of inhalation of SO_2 at all concentrations tested. The decrease in compliance was not large, and occurred after 10-15min SO_2 inhalation."
"The trachea, bronchi, and lungs contained the majority of the S^{35} given as $S^{35}O_2$ since it was administered via inhalation via trachea cannulation. However, the hilar lymph nodes, and in one instance the abdominal lymph nodes, contained a considerable proportion of the S^{35} retained, considering their size."
"SO_2 is quickly and readily absorbed and the arterial blood level rises quickly after the onset of SO_2 absorption, but soon begins to fall after the end of exposure as diffusion into the tissues occurs."

Review – Assessment:

A. Test animals:	±✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	±✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* ±✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* +✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.*

	-✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* ±✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? -✎ Is the control regime appropriate? ±✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? ±✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective?

	+✏ Were assumptions reasonable? +✏ Are the methods reproducible? +✏ Did either the study design or the statistical analysis limit confounding variables? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings demonstrate that SO_2 does have respiratory effects in dogs, even at relatively low concentrations, and decreases compliance and increases airway resistance. Also showed that $^{35}SO_2$ when inhaled was absorbed quickly, distributed widely to many tissues and readily gained access to many tissues, including the brain. The results are similar to those seen with guinea pigs, rats and mice. Do not warrant any extrapolations to SO_2 effects on tissue structure. No mention of extrapolating results to human responses. Given that this study was done in 1960, these findings would have been relatively novel.

Confidence index:

This paper was written in 1960, and the lack of detailed methods, and slightly questionable techniques by today's standards seem to be more a product of the time than a lack of care or expertise involved in conducting the study. Did not adequately reflect characteristics of dogs, possibly confounding results. For example, strain, age and sex of dogs was not given. In addition, it did not provide 5 animals per dose level. While it did account for nominal and actual exposure levels, description of pre-exposure conditions, including acclimation period were not given. Furthermore, no details were given regarding pre-experimental conditions including what dogs ate or whether they were acclimatized. Extrapolation that effect of long continued low exposure to SO_2 may effect tissue structure based on its distribution is not warranted. No morphological data on effects, only concentration of $^{35}SO_2$ present in each tissue. Data for pulmonary function and distribution patterns are in agreement with other published works, which used guinea pigs, rats and mice.

Review – Confidence index ranking:

Low

Author: Fairchild, GA.	**Internal ID:** 238
Title: Effects of Ozone and Sulfur Dioxide on Virus Growth in Mice	
Year: 1977	
Abstract: Inhalation of ozone and sulfur dioxide inhibited influenza virus growth in the nose of mice. Ozone inhalation caused the more pronounced inhibition of influenza virus growth: 0.6 ppm ozone for 3 hours post-virus exposure almost completely inhibited influenza virus growth in the nose, whereas sulfur dioxide (6 ppm for 7 days) caused only partial inhibition of influenza growth in the nose. Neither gas altered the propagation of influenza virus in the lungs of mice. Vesicular stomatitis virus (VSV) growth was either unaffected by exposure to ozone or, when ozone exposure preceded VSV exposure, the virus may have grown to slightly higher titer. The inhibitory effect of ozone and sulfur dioxide on influenza virus growth in nasal epithelium suggests a competitive interaction between the chemical inhalant, the virus, and host tissues, with net consequences for the pathogenesis of this disease. If the effects of these inhalants are to be properly interpreted, they should be determined for all major regions of virus growth and inhalant deposition.	
Objective: To demonstrate the effects of ozone and sulfur dioxide on the growth of influenza virus and vesicular stomatitis virus in the nose and lung of mice.	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
6 ppm	7 days	mice	CD-1	7 to 8 weeks	male	unknown	Pathogen-free	unknown	unknown

Observations:

Ozone inhalation caused the more pronounced inhibition of influenza virus growth: 0.6 ppm ozone for 3 hours post-virus exposure almost completely inhibited influenza virus growth in the nose, whereas sulfur dioxide (6 ppm for 7 days) caused only partial inhibition of influenza growth in the nose. Neither gas altered the propagation of influenza virus in the lungs of mice. Vesicular stomatitis virus (VSV) growth was either unaffected by exposure to ozone or, when ozone exposure preceded VSV exposure, the virus may have grown to slightly higher titer.
*Statistically significant

Author's Conclusions:

"The inhibitory effect of ozone and sulfur dioxide on influenza virus growth in nasal epithelium suggests a competitive interaction between the chemical inhalant, the virus, and host tissues, with net consequences for the pathogenesis of this disease. If the effects of these inhalants are to be properly interpreted, they should be determined for all major regions of virus growth and inhalant deposition."

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+/-) c) Sex (+) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (+) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective?

	(-) Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? N/A Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*

F. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	N/A Are the statistical methods used appropriate? (-) Are confidence intervals reported? N/A Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study showed that SO_2 exposure following viral infection was able to partially inhibit influenza replication in the nose of mice but had no effect on virus growth in the lungs as compared with control group. Previous experiments of a similar nature have demonstrated conflicting results of the effect of SO_2 on the pathogen of viral infection. Results from this study suggest that SO_2 and the virus may compete for host tissues with net consequences for the disease pathogenicity. The recognition of these inhibitory effects and the determination of the mechanism of action may be important in the investigation of the clinical severity of disease, which will depend on the total viral antigen in the entire respiratory.

Confidence index:

This study, while describing many of the characteristics of the animals involved, did not mention number of animals. Consequently, it is unclear whether results should be considered statistically significant. Pre-exposure conditions were not described and no explicit rationale was given for the exposure level. In addition, chamber temperature and humidity levels were not defined. Mechanism of inhibiting viral growth is not defined; this must be known before any conclusions or extrapolations can be made. Data are in agreement with another published study in mice that showed SO_2 at similar levels was able to inhibit (by 5%) pneumonia. No statistical analysis was performed to try and validate the observations. Exposure conditions were not well defined, but experimental details for viral concentration were.

Review – Confidence index ranking:

Moderate to low

Author: Davies A, Dixon M, Penman R, Widdicombe JG, Wise JCM	**Internal ID: 239**
Title: Effect of repeated exposures to high concentrations of sulfur dioxide on respiratory reflexes in rabbits.	
Year: 1978	
Abstract: Rabbits were exposed to sulfur dioxide, either 300ppm for 3h or 150ppm for 12 3-hour periods. After exposure we measured (1) lung mechanics, (2) Breuer-Hering inflation reflex, (3) breathing responses to inhaled carbon dioxide and ammonia, pneumothorax and intravenous injections of histamine and phenyl diguanide, and (4) activity of pulmonary stretch and lung irritant receptors by single-fibre recording. The results indicated that chronic exposure to SO_2 inhibits pulmonary stretch receptor activity and its reflex effects, and raises the question whether this mechanism may play a part in the control of breathing in human patients with chronic bronchitis.	
Objective: "In this paper we describe experiments designed to test the effects of chronic exposure of rabbits to SO2 on lung reflex control of breathing, in the hope that results might be relevant to chronic bronchitis. We have also studied changes during a recovery period after exposure."	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
150 or 300ppm	**300ppm – 3hrs 150ppm – 12x3hrs**	**Rabbit**	**New Zealand white**	**Adult (3-4.2kg)**	**M & F**	**50**	**n/a**	**n/a**	**150ppm**

Observations:

Three days after exposure to 300ppm SO_2 for 3hrs, treated animals had a significantly higher lung resistance than a group of control animals ($p<0.01$), while no differences were observed for those treated with 150ppm for twelve 3hr periods.

Breathing frequency decreased in both 300ppm and 150ppm treated animals, but recovered after 1 hr in 300ppm animals, while it stayed decreased in 150pppm animals even at one hour.

The Breuer-Hering inflation reflex decreased in multiple low dose (150ppm) exposed animals ($p<0.01$).

Single dose (150ppm) exposed animals showed a plateau in the response of stretch receptors at higher lung volumes, while those exposed to multiple-low doses showed no effect.

*Statistically significant

Author's Conclusions:

"Rabbits were chronically exposed to sulfur dioxide, to an extent that sometimes increased total lung resistance to airflow, but produced no histological damage in the tracheobronchial epithelium apart from a disappearance of cilia. This treatment reduced the strength of the Breuer-Hering reflex, and produced changes in the pattern of breathing consistent with this effect. Recordings of single-fibre activity from pulmonary stretch receptors showed that their sensitivity was reduced. Breathing responses to pneumothorax and to inhaled carbon dioxide or ammonia also suggested that pulmonary stretch receptors and Breuer-Hering reflex activity was depressed. Other lung reflexes, such as those induced by intravenous injections of histamine or phenyl diguanide possibly acting on lung J-receptors, were little affected. The results raise the question whether the changes in the nervous control of breathing in SO_2-induced chronic bronchitis in rabbits may apply also to other forms of chronic bronchitis, including that in man."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* +b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated?

	☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? -✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? +✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +✎ Are the observed trends statistically significant?

H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The study showed that multiple low dose exposure to SO_2 can inhibit pulmonary stretch receptors in rabbits. Because this inhibition can act to increase bronchoconstriction, the role of stretch receptors in human chronic bronchitis is suggested. This extrapolation may not be warranted because recovery of rabbits following SO2 exposure was 7 days - does not apply to human chronic bronchitis so other mechanisms may also be different.

Confidence index:

The study appears to have been quite thorough, examining a wide variety of respiratory function parameters, even though follow-up was restricted to 7 days. This study was also limited in its description of the materials and methodology used; no mentions of pre-trial conditions and whether animals were acclimatized prior to the study are made. Furthermore, food type and amount available to rabbits is not specified. Still, sample size is appropriate (n=50) and good controls were used. Exposure conditions and assessment methods were well defined – study should be easily reproducible. Multiple parameters were measured to asses lung function. Data are in agreement with other published works which suggest that SO_2 inhibits activity of pulmonary stretch receptors, however, does not warrant extrapolation to human chronic bronchitis.

Review – Confidence index ranking:

Moderate to high

Author: Drew RT. Kutzman RS. Costa DL. Iwai J.	**Internal ID: 241**
Title: Effects of sulfur dioxide and ozone on hypertension sensitive and resistant rats.	
Year: 1983	
Abstract: Two lines of Dahl rats, one resistant to salt-induced hypertension (DR) and one susceptible to salt-induced hypertension (DS) were subchronically exposed to SO_2 (50 ppm, 6 hr/d, 5 d/wk for 31 weeks) or ozone (2.0 ppm, 6 hr/d, 5 d/wk for 20 weeks). Subgroups of rats were maintained on either high or low salt diets. In rats not expected to develop hypertension, exposure to SO_2 caused a slight but consistent decrease in blood pressure. In DS rats on a high salt diet exposure to SO_2 resulted in an increase in blood pressure over that of their air exposed counterparts. All exposure-related differences in blood pressure disappeared after the last exposure to SO_2. Exposure to ozone was fatal to all DS rats, regardless of the amount of salt in the diet. The DR rats were more resistant to ozone, with most animals surviving the 20-week exposure. Ozone-exposed rats exhibited a decrease in both growth rate and blood pressure in all groups when compared to their air-exposed counterparts. It is not known if exposure-related blood pressure differences would persist after ending ozone exposures. After brief exposures, ozone caused increased lung weights in both groups, but there were no consistent changes in pulmonary nonprotein sulfhydryl groups. Hepatic nonprotein sulfhydryl levels were consistently, but not significantly, lower in ozone-exposed rats.	
Objective: "These studies were designed to investigate the effects of three variables, rat line, dietary salt, and exposure to air pollutants on growth, blood pressure, and mortality."	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
50ppm	**6hr/d 5d/wk for 31wks**	**Rat**	**Dahl**	**21d**	**Male**	**40 DR** **40 DS**	**Resistant (DR)/ susceptible (DS) to salt induced hypertension**	**n/a**	**50ppm**

Observations:

"The growth rates of all groups of SO_2 exposed animals were slightly, but significantly ($p<0.01$) less that that of their exposed counterparts parts. The initial blood pressures in DS animals were always markedly higher ($p<0.01$) than in DR animals (128(±1.3) and 112(±2.0)mmHg, respectively for DS and DR rats on low salt diets and exposed to air). The mean blood pressure of DS animals maintained on high salt diets increased as expected. Except for the DS rats on high salt diets, exposure to SO_2 caused a slight, but statistically significant ($p<0.01$) reduction in blood pressure. However, the SO_2-exposed DS rats on high salt diets had blood pressures significantly higher ($p<0.01$) than those of their air-exposed counterparts. Blood pressure differences between the two groups exposed to air or SO2 disappeared after the final exposure."
*Statistically significant

Author's Conclusions:

"These investigations were biased toward producing an interaction because the levels of dietary salt and pollutant exposure were both chosen to produce marked changes. In the SO_2 study, the reduction in growth demonstrated that the SO_2 concentration selected (50ppm) was high enough to produce an effect. The only positive interaction resulting in higher blood pressure was seen with DS rats on high salt diets exposed to 50ppm SO_2. In the other three groups, SO_2 exposure resulted in a slight but statistically significant decrease in blood pressure. The mechanism for these changes is not known and may not be direct effect of SO_2. These differences rapidly disappeared after the exposures were terminated. Ambient levels of SO_2 rarely exceed 0.5ppm, even during episodes of high air pollution. Since these studies were conducted at over 100times ambient levels of SO_2, and since there were only slight variations in blood pressure, it seems reasonable to conclude that there is little chance of an influence of ambient levels of SO_2 on hypertension."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ±✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	+✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers

	appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* +✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* +✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* +✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? +/-✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* ±✎ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? ±✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate?

	±✓ Are confidence intervals reported?
	+✓ Did the investigator appropriately interpret the statistics?
	+✓ Are the observed trends statistically significant?
H. Interpretations:	+✓ Was the original objective addressed?
	+✓ Are the results novel?
	+✓ Was the design of the study appropriate to investigate the primary objective?
	+✓ Were assumptions reasonable?
	+✓ Are the methods reproducible?
	+✓ Did either the study design or the statistical analysis limit confounding variables?
	+✓ Do the conclusions make biological sense?
	+✓ Are the extrapolations reasonable?
	+✓ Is the paper in a peer-reviewed journal?
	+✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study observed that in rats susceptible to salt induced hypertension that were on a high salt diet, exposure to SO2 resulted in an increase in blood pressure over air exposed counterparts. In rats not expected to develop hypertension, exposure to SO_2 caused a slight but consistent decrease in blood pressure. All exposure related differences in blood pressure disappeared after the last exposure to SO_2. Since the level of SO_2 used is 100 times that found in ambient air it suggests that SO_2 may not have an influence on hypertension – insufficient data to warrant an extrapolation to humans.

Confidence index:

Sufficient sample size to identify statistically significant changes in blood pressure. Used same age and sex animals – tried to limit confounding factors. Only two endpoints were measured – weight and blood pressure, additional parameters associated with hypertension should have been investigated. Also should have looked at parameters that SO_2 is known to affect ie lung mechanics. Although measurements were made after 4 week exposure and every other week out to 31 weeks no raw data was shown, no graph to see trends only data reported at 31 weeks. No justification for the single dose used, no references cited for similar studies performed in humans – yet extrapolations to humans were attempted.

Review – Confidence index ranking:

Low to moderate

Author: Alarie Y, Wakisaka I, Oka S.	**Internal ID: 243**

Title: Sensory irritation by sulfur dioxide and chlorobenzilidene malonitrile.

Year: 1973

Abstract: Sulfur dioxide and o-chlorobenzilidene malonitrile were shown to initiate sensory irritation of the upper respiratory tract in mice. With sulfur dioxide the reaction was accompanied with desensitization within a few minutes of continuous exposure while with o-chlorobenzilidene malonitrile the sensory irritation persisted at the same level during the entire exposure period. The desensitized state related to sulfur dioxide exposure was specific for this agent. Mice desensitized to sulfur dioxide reacted o-chlorobenzilidene malonitrile as control mice not previously exposed to sulfur dioxide. The degree of sensory irritation by sulfur dioxide was linearly related to the logarithm of the concentration, whereas the time for maximum reaction to occur varied inversely with that factor. A sequence of events for sensory irritation and desensitization is suggested based on the reducing properties of sulfite toward disulfide bonds in a receptor protein.

Objective: To examine sensory irritation and desensitization processes, and to compare the action of SO2 with other sensory irritants, such as o-chlorobenzilidene malonitrile.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0-298ppm	**10min single or 10 min repeated**	**Mice**	**Swiss albino**	**Adult 22-24g**	**Male**	32 + 12	**n/a**	**n/a**	**17ppm**

Observations:

"A rapid decrease in respiratory rate occurred following initiation of exposure at each concentration; the rapidity of onset and depth of respiratory depression was apparently related directly to the concentration of SO_2. The linear dose-response relationship obtained when the maximum decrease at each exposure level was plotted against the logarithm of the exposure concentration, substantiates that the maximal depression of respiration is a function of SO_2 concentration within the exposure limits explored."

"…respiratory depression was followed by a return to the control respiratory rate even while exposure to SO_2 continued. The rate of recovery did not seem to be affected by the concentration of SO_2."

"When simultaneous exposures to both o-chlorobenzilidene malonitrile at 75μg/l air and SO_2 at 100ppm were made… no change in respiratory pattern indicating a recovery … was observed, the response being characteristic of o-chlorobenzilidene malonitrile alone."

"exposure of mice to SO_2 through a tracheal cannula in order to bypass the upper airways produced no decrease in respiratory rate during the first half of the 10min exposure; the respiratory pattern was totally undisturbed."

*Statistically significant

Author's Conclusions:

"The results indicate that maximal sensory irritation of the upper respiratory tract by SO_2, as indicated by the depression in respiratory rate, occurred within 1-2min in mice. The magnitude of maximal depression varied directly with the logarithm of the SO_2 exposure concentration while the time for its appearance varied inversely with that factor. Following maximal response, a return toward control values suggested the disappearance of sensory irritation despite continued exposure. The return toward control values while exposure to SO_2 continued may be designated "desensitization."

"When o-chlorobenzilidene malonitrile and SO_2 were given simultaneously the reaction observed was characteristic of the reaction of animals exposed to o-chlorobenzilidene malonitrile alone in which no sign of desensitization by SO_2 was observed. Thus, the action of the two materials was at different sites, each acting independently."

Review – Assessment:

A. Test animals:	-✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective?

	-✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* +✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+/-✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? +/-✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? -✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? -✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical?

	+✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? ±✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics? +/-✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Sulphur dioxide was shown to initiate sensory irritation of the upper respiratory tract in mice. With sulfur dioxide the reaction was accompanied with desensitization within a few minutes of continuous exposure. This phenomenon was also observed by repeated brief exposures to SO_2 with short recovery periods between each exposure. Proposal the SO_2 can desensitize cholinergic nerve endings are supported by other published works.

Confidence index:

This experiment used an assortment of different groupings of mice. None of the groups were big enough to produce statistically significant results, most consisting of just 4 mice per group. Furthermore, possible confounding factors such as age and pre-trial health were not given. Rationale behind concentration levels of SO_2 administered to mice was not explained, and no mention was made of preliminary trials to come up with these exposure levels. Furthermore, pre-trial conditions such as amount and type of food used and climatic conditions of the environment, or acclimation period was not given. Assumption that decrease in respiratory rate signifies stimulation of nasal trigeminal stimulation is not warranted – nevertheless authors do provide a potential mechanism by which SO_2 can cause desensitization of cholinergic nerve endings. Exposure conditions and assessment method were well defined – study should be easily reproducible. Single endpoint of respiratory rate was used – additional parameters including morphological or biochemical changes associated with stimulation of nasal trigeminal nerve endings would have helped validate the data. Conclusion that SO_2 is able to desensitize cholinergic receptors is consistent with other published works. Did try to limit confounding factors by using same sex and weight mice, each mouse served as its own internal control.

Review – Confidence index ranking:

Moderate to low

Author: Davenport, PW., Freed, AN. and Rex, KA. **Internal ID:** 244

Title: The Effect of Sulfur Dioxide on the Response of Rabbits to Expiratory Loads

Year: 1984

Abstract: The role of slowly adapting pulmonary stretch receptors in the reflex control of expiratory duration was investigated in anesthetized, spontaneous breathing rabbits. Graded resistive and elastic loads were applied for single expirations to produce expiratory volume-time curves. The PSR were selectively blocked by administration of 200 to 400 ppm sulfur dioxide to the inspired air of the rabbits. Volume-time curves were generated for pre-treatment, SO_2 exposure, recovery and post-bilateral cervical vagotomy. SO_2 exposure significantly reduced the volume-dependent modulation of expiratory duration from the pre-treatment levels. The response to the expiratory lads returned when the SO_2 gas was removed. Vagotomy completely abolished any volume-dependent modulation of expiratory duration with expiratory loading. These results demonstrate that the response of rabbits to expiratory loads is vagally dependent. The hypothesis that PSR are the vagal afferents mediating this response is supported by these results.

Objective: To demonstrate the effect of sulfur dioxide on the response of rabbits to expiratory loads.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
200 to 400 ppm	15 to 20 minute	rabbits	New Zealand white	unknown	unknown	14	unknown	unknown	unknown

Observations:

The volume of air expired during a loaded breath decreased with increasing load magnitude, resulting in a concomitant increase in T_E for both resistive and elastic loads. When the rabbit was exposed to SO_2 for 15-20 min, the breathing frequency decreased as a result of a significantly increased T_I . T_E was not significantly different from the control and the tidal volume was also significantly increased. There was a significant increase in the V_E-T_E slope after SO_2 exposure, the greatest increase being associated with the higher concentrations of SO_2. The T_E values for each load level were significantly greater than the T_E for unloaded breaths, indicating that the expiratory loading response was not completely abolished by SO_2. Bilateral cervical vagotomy resulted in a significant increase in the unloaded T_I, T_E and tidal volume from all other treatments. In particular, these changes were significantly increased from the SO_2 levels.

*Statistically significant

Author's Conclusions:

"These results demonstrate that the response of rabbits to expiratory loads is vagally dependent. The hypothesis that PSR are the vagal afferents mediating this response is supported by these results."

"These experiments infer a nearly complete abolition of PSR activity at 400 ppm concentrations of SO_2 in rabbits. The resultant abolition of the vagally-mediated volume-dependent modulation of TE with resistive and elastic loading provides strong evidence for PSR being the mediators of the peripheral control of T_E."

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+/-) c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary

	objective? (-) Was evidence provided to justify the dosing regime? (-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+/-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+/-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (-) Were test animals randomly assigned to exposure groups? (+/-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+/-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*

F. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+/-) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+). Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The present study demonstrates that SO_2 abolition of PSR activity mediated the volume dependent modulation of expiratory duration and provides strong evidence for PSR being the mediators of the peripheral control of expiratory duration. Data cannot be extrapolated to human responses as rabbits are the only species known to have PSR inhibited by SO_2. Consequently, extrapolation of study findings to other species would be difficult. Results support the hypothesis that PSR are the vagal afferents that mediate this response.

Confidence index:

This study outlines some of the characteristics of rabbits involved but does not indicate gender or pre-trial health. It is not mentioned whether rabbits were acclimatized prior to the exposure or what conditions were within the exposure chamber. Furthermore, no mention was made of type or quantity of feed used. Exposure conditions and assessment methods were straightforward (small lack of details about specific dose and time ie 200-400 ppm, 15-20 min). Consequently, the study would be easily reproducible.

Review – Confidence index ranking:

Moderate

Author: Halinen AI. Salonen RO. Pennanen AS. Kosma VM.	**Internal ID: 245**

Title: Combined respiratory effects of cold air with SO(2) or NO(2) in repeated 10-minute exposures of hyperventilating guinea pigs.

Year: 2000

Abstract: Previous studies in asthmatic subjects and guinea pigs have demonstrated attenuation of bronchoconstriction in repeated exposures to clean cold dry air. In the present animal study, we have simulated short-lasting human exposures to subfreezing urban air containing sulfur dioxide (SO(2)) and nitrogen dioxide (NO(2)). The anesthetized, paralyzed, and mechanically ventilated guinea pigs had 4 consecutive 10-min exposures either to clean cold dry air or to cold air with graded concentrations of SO(2) (0-5 ppm) or NO(2) (0-4 ppm). Peak expiratory flow (PEF) and tidal volume (V(T)) were continuously measured both during and after highly controlled exposures. Bronchoalveolar lavage fluid (BALF) and histological samples were obtained after finishing the consecutive exposures. Cold air + SO(2) at 1 and 2.5 ppm (n = 12) produced immediate concentration-dependent increases in the lung function responses compared to the preceding single exposure to clean cold dry air in the same animals (DeltaPEF = -32.7 +/- 6.1% and -35.6 +/- 6.5% vs. -27.0 +/- 3.1%; DeltaV(T) = -22.4 +/- 4.4% and -28.3 +/- 4.7% vs. -18.1 +/- 2.9%). In a multivariate analysis, these responses were significantly larger than the attenuated lung function responses to the corresponding second and third clean cold dry air exposures (p <. 05). The fourth exposure to cold air + SO(2) at 5 ppm produced a smaller response (DeltaPEF = -25.3 +/- 4.8% and DeltaV(T) = -17.8 +/- 3.7%) than cold air with the lower SO(2) concentrations. Cold air + NO(2) at 1 and 2.5 ppm (n = 12) produced roughly similar lung function responses to the preceding single exposure to clean cold dry air in the same animals, and there was no significant attenuation of bronchoconstriction as with the consecutive exposures to clean cold dry air. The largest decreases in lung functions (DeltaPEF = -33.8 +/- 6.7% and DeltaV(T) = -26.2 +/- 6.8%) were recorded during the fourth exposure, which was to cold air + NO(2) at 4 ppm. In the cold air + SO(2) group, there was a significantly lower proportion of macrophages in the differential count of BALF white cells compared to the clean cold dry air group. In addition, there was eosinophilic infiltration within and below the tracheal epithelium in all guinea pigs exposed to either clean cold dry air, cold air + SO(2), or cold air + NO(2). In conclusion, the addition of moderate concentrations of SO(2) or NO(2) to clean cold dry air counteracted the attenuation of bronchoconstriction induced by repeated cold dry air exposures in guinea pigs. Cold air + SO(2) also decreased the proportion of macrophages in BALF white cells.

Objective: "The aims of this study were: 1) to simulate short-lasting human exposures to subfreezing urban air containing SO_2 and NO_2 in a recently developed guinea pig model, and 2) to apply physiological, cytological, and morphological methods for the characterization of respiratory responses to these combined exposures."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0-5ppm	4x10min consecutive	Guinea pig	Dunkin-Hartley	n/a	Male	12 + 15 control	n/a	n/a	1ppm

Observations:

"Cold air+SO_2 at 1 and 2.5ppm produced small concentration-dependent increases in the (bronchoconstriction) responses compared to the preceding clean cold dry air exposure in the same animals. During the fourth exposure to cold air + SO_2 at 5ppm, the lung function response was about the same as during the first clean cold dry air response."

No statistically significant differences were noticed in lung function (PEF or V_T) decreases between the four recovery periods.

Blood pressure also increased following exposure to cold dry air + 5ppm SO_2
From the BALF, proportion of macrophages was lower in the SO_2 exposed group, while proportions of lymphocytes and eosinophils trended toward being higher.

Author's Conclusions:

"We showed that the addition of graded concentrations of SO_2 (1 and 2.5ppm) or NO_2 (1-4ppm) into cold dry air enhanced the lung function responses of guinea pigs in the present repeated exposures. However, the exposure to cold+SO_2 at 5ppm produced an attenuated lung function response compared to cold air with the lower SO_2 concentrations. The guinea pigs had also weak post-exposure bronchoconstrictions after all exposure periods, but these responses seemed to be unrelated to the actual exposure condition."
"The present results indicated that the addition of moderate concentrations of SO_2 (1 and 2.5ppm) or NO_2 (1-4ppm) to clean cold dry air counteracted the attenuation of bronchoconstriction induced by repeated cold dry air exposures in guinea pigs. Cold air + SO_2 also decreased the proportion of macrophages in BALF white cells. Finally, the pathophysiological effects of combined exposures to cold air + SO_2 and cold air + NO_2 should be tested further in experimental setups using longer exposure durations."

Review – Assessment:

A. Test animals:	+✏ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✏ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✏ Were the chosen exposure levels appropriate to investigate the primary objective? +/-✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✏ Were both nominal and actual exposure concentrations recorded? +✏ Was the duration of exposure appropriate to investigate the primary objective?

	+✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +/-✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? +✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)?

	+✓ Were accepted assessment methods employed? +✓ Were assessment measures sufficiently sensitive? +✓ Was the nature and severity of symptoms graded against an explicitly defined scale? +✓ Did qualified personnel conduct the exams? +✓ Was the timing and frequency of assessment logical? +✓ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✓ Was individual data reported for each test animal?
G. Data analysis:	+✓ Are the statistical methods used appropriate? +✓ Are confidence intervals reported? +✓ Did the investigator appropriately interpret the statistics? +✓ Are the observed trends statistically significant?
H. Interpretations:	+✓ Was the original objective addressed? +✓ Are the results novel? +✓ Was the design of the study appropriate to investigate the primary objective? +✓ Were assumptions reasonable? +✓ Are the methods reproducible? +✓ Did either the study design or the statistical analysis limit confounding variables? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The study demonstrated that repeated exposures to cold dry polluted air (SO_2 or NO_2) reduce the attenuation in bronchoconstriction that occurs after repeat exposures after cold dry air alone, however, some attenuation does still occur. Interestingly, higher levels of SO_2 (5ppm) actually show a reduced lung response that 1 or 2.5ppm SO_2. This result was accompanied by a decrease in the proportion of macrophages in BALF white cells. No extrapolations were made to responses of asthmatics and short exposures to cold dry air.

Confidence index:

Sufficient sample size to identify statistically significant results. Differential counts of white blood cells in bronchoalveolar lavage fluid were given in percentages – unknown if the number cells recovered from each animal was consistent. Each guinea pig served as its own internal control, as well a single exposure to cold dry air was given prior to repeated exposures to SO_2 and comparisons were made to this initial preexposure value, also an intravenous injection of propranolol was given to block all possible bronchodilator effects of endogenous adrenaline and to enhance the overall responsiveness of the tracheobronchial airways to exogenous stimuli – attempt to limit confounding factors. Exposure conditions and assessment methods were well defined – study should be easily reproducible. Multiple parameters were assessed concerning both lung function and cytology. Did mention using morphological analysis but no data presented. Some results do contradict some previously published works, others support previously published observations.

Review – Confidence index ranking:

Moderate to high

Author: Halinen, A.I., Salonen, R.O., Pennanen, A.S., Kosma, V.M. **Internal ID:** 246

Title: Combined Respiratory Effects of Cold Air with SO_2 or NO_2 in Single 1-hour Exposures of Hyperventilating Guinea Pigs

Year: 2000

Abstract: The present study is a continuation of our previous experiments on repeated 10-min exposures of anesthetized, mechanically ventilated guinea pigs to clean cold dry air (Halinen et al., 2000a), and to cold air plus gaseous air pollutants (Halinen et al., 2000b). This time we made continuous 60-min exposures to clean cold dry air, cold air + SO_2 at 1 ppm, cold air + NO_2 at 1 ppm, and warm humid air + NO_2 at 1 ppm, and focused on responses at 10-60 min. Clean cold dry air and cold air + pollutants (n = 8-9 in each group) produced similar cooling in the guinea pig lower respiratory tract. The decreases in intratracheal temperature (T_{tr}) reached a plateau at 20 min with mean maximal decreases of 9.7-11.3 degrees C from the pre-exposure control values of 36.0-37.3 degrees C. In contrast, there were progressive decreases in esophageal temperature (T_{oe}) during the exposures, indicating constant conductive and evaporative heat losses from the tracheobronchial tissues. The mean maximal decreases in T_{oe} were 1.2-1.4 degrees C from the preexposure control values of 37.8-38.0 degrees C. Clean cold dry air induced 4. 5-10.8% mean decreases in peak expiratory flow (PEF) at 10-60 min of exposure, which were statistically nonsignificant due to a relatively large variation between animals. Cold air + SO_2 at 1 ppm induced a mean decrease of 11.4% in PEF at 10 min ($p <.05$), which was spontaneously abolished during the next 10 min of exposure. Cold air + NO_2 at 1 ppm caused no decrease, but in fact small, nonsignificant increases in PEF at 30-60 min of exposure. Cold air + NO_2 at 1 ppm, and to some extent also cold air + SO_2 at 1 ppm, attenuated significantly the mechanical ventilation induced gradual decrease in tidal volume (V(T)), when compared to clean cold dry air exposure. Cold air + NO_2 at 1 ppm, but not warm humid air + NO_2 at 1 ppm, increased significantly the proportion of macrophages in the differential count of bronchoalveolar lavage fluid (BALF) white cells when compared to both clean warm humid air and cold dry air. None of the exposure conditions caused morphological or inflammatory changes in the respiratory tissues. In conclusion, continuous 60-min exposures to clean cold dry air, cold air + SO_2, and cold air + NO_2 produced weaker functional effects on the lower respiratory tract of guinea pigs than our previous consecutive 10-min exposures to these air conditions. After the first 10 min, there was a strong attenuation of the bronchoconstrictor responses, especially to cold air + NO_2 at 1 ppm. The small airway effects of prolonged mechanical ventilation were significantly modified by NO_2 at 1 ppm in both cold dry and warm humid breathing air. Finally, cold air + NO_2 at 1 ppm increased the proportion of macrophages in BALF white cells.

Objective: To assess the combined respiratory effects of cold air with SO_2 or NO_2 in single 1-hour exposures of hyperventilating guinea pigs.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
1 ppm SO_2	1hour continuous	Guinea pigs	Dunkin-Hartley	No info	Male	8 and 8 in control and each of the other exposure groups (NO_2)	**No info**	N/A	N/A

Observations:

Clean cold dry air and cold air + pollutants (n = 8-9 in each group) produced similar cooling in the guinea pig lower respiratory tract. The decreases in intratracheal temperature reached a plateau at 20 min with mean maximal decreases of 9.7 – 11.3°C from the pre-exposure control values of 36.0 – 37.3°C. In contrast, there were progressive decreases in esophageal temperature during the exposures, indicating constant conductive and evaporative heat losses from the tracheobronchial tissues. The mean maximal decreases in T were 1.2 – 1.4 C from the preexposure control values of 37.8 – 38.0°C. Clean cold dry air induced 4.5-10.8% mean decreases in peak expiratory flow at 10-60 min of exposure, which were statistically nonsignificant due to a relatively large variation between animals. Cold air + SO_2 at 1 ppm induced a mean decrease of 11.4$ in PEF at 10 min, which was spontaneously abolished during the next 10 min of exposure. Cold air + NO_2 at 1 ppm caused no decrease, but in fact small, nonsignificant increases in PEF at 30-60 min of exposure. Cold air + NO_2 at 1 ppm, and to some extent also cold air + SO_2 at 1 ppm, attenuated significantly the mechanical ventilation induced gradual decrease in tidal volume, when compared to clean cold dry air exposure. Cold air + NO_2 at 1 ppm, but not warm humid air + NO_2 at 1 ppm, increased significantly the proportion of macrophages in the differential count of bronchoalveolar lavage fluid white cells when compared to both clean warm humid air and cold dry air. None of the exposure conditions caused morphological or inflammatory changes in the respiratory tissues.

Author's Conclusions:

Continuous 60-min exposures to clean cold dry air,cold air + SO_2, and cold air + NO_2 produced weaker functional effects on the lower respiratory tract of guinea pigs than our previous consecutive 10-min exposures to these air conditions. After the first 10 minutes, there was a strong attenuation of the bronchoconstrictor responses.

**conclusions primarily deal with the NO_2 exposure

Review – Assessment:

A. Test animals:	(+)Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+)a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-)b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+)c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source Guideline: An established breeding facility or educational institution

B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (+)Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-)Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water May be withheld optionally.*
D. Equipment:	(+/-)Was information pertaining to the type, dimensions, material and

	atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+)Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-)Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random Assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+/-)Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+)Were appropriate endpoints examined? (+/-)Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
H. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+/-)Was the design of the study appropriate to investigate the primary

	objective? (+/-)Were assumptions reasonable? (+)Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study showed that a continuous 60-min exposure to clean cold dry air + 1ppm SO_2 produced weaker functional effects on the lower respiratory tract in guinea pigs than their previous study using consecutive 10-min exposures. This regimen also did not produce any significant changes in the cell distribution of bronchoalveolar lavage fluid. No extrapolations were made to responses of asthmatics and short exposures to cold dry air, even though this model was designed to simulate usual human exposures in urban environments to cold air and gaseous pollutants.

The animals were mechanically ventilated which allows control over the breathing and forced mouth breathing. However, this also limits the comparability and extrapolaton of the results. The forced mouth breathing was used due to the assumption that "some humans are mouth breathers even at rest and all humans increase their mouth breathing while increasing the level of exercise". None of the exposure conditions caused morphological or inflammatory changes in the tracheobronchial airways or lung tissue of guinea pigs. Only cold air +SO_2 at 1ppm showed a statistically significant bronchoconstrictor response at 10 min, which was spontaneously abolished during the next 10 min of exposure. This may indicate a possible adaptive mechanism. The exposure level used is more typical of a potential human exposure.

Confidence index:

This study recorded animal characteristics such as strain and sex, but omitted other factors such as age and health of animals that might have confounded results. Furthermore, no mention was made of pre-trail conditions, or food type or amount. It was also unclear as to how animals were divided into groups and whether this process was random or not.

Differential counts of white blood cells in bronchoalveolar lavage fluid were given in percentages – unknown if the number cells recovered from each animal was consistent. Each guinea pig served as its own internal control, as well an intravenous injection of propranolol was given to block all possible bronchodilator effects of endogenous adrenaline and to enhance the overall responsiveness of the tracheobronchial airways to exogenous stimuli. Exposure conditions and assessment methods were well defined – study should be easily reproducible. Multiple parameters were assessed concerning both lung function and cytology. Did mention using morphological analysis but no data presented. Some results do contradict some previously published works, others support previously published observations.

Review – Confidence index ranking:

Moderate

Author: Haider SS. Hasan M. Khan NH.	**Internal ID: 249**

Title: Air pollutant sulfur dioxide-induced alterations on the levels of lipids, lipid peroxidation and lipase activity in various regions of the rat brain.

Year: 1982

Abstract: The exposure of rats to SO_2 (10 p.p.m.) for one hour daily for 30 days caused depletion of total lipids in all brain areas. The contents of phospholipid were elevated in cerebellum and brain stem, but were depleted in cerebral hemisphere. Cholesterol levels showed an increase in various brain regions. On the other hand, gangliosides were increased in cerebellum and brain stem, but were decreased in cerebral hemisphere. Interestingly, cholesterol/phospholipid ratio was increased in different regions of the brain. Lipase activity was elevated in cerebral hemisphere. Lipid peroxidation showed marked increment in whole brain and in all the brain areas studied. The results suggest that SO_2-exposure induces degradation of lipids. Interestingly, the lipid contents are affected differentially in the various parts of the brain.

Objective: To examine lipid profiles and biochemical parameters in the brain following SO_2 exposure.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
10ppm	1h/d for 30d	Albino Rat	Charles-Foster	Adult 150-200g	Male	12	n/a	n/a	10ppm

Observations:

"A statistically significant decrement in total lipid contents in all areas of the brain was found ($p<0.02$, $p<0.001$, and $p<0.001$). The levels of phospholipids were increased in the cerebellum ($p<0.001$) and in brain stem, the increase was only 8%, cerebral hemisphere exhibited depletion of only 6%."

Cholesterol concentration increased in cerebral hemisphere and cerebellum ($p<0.05$, $p<0.001$) but elevation in brain was only 14%.

Gangliocides were elevated in the cerebellum and brain stem ($p<0.001$), but diminished in cerebral hemisphere.

Lipase activity was significantly increased in cerebral hemisphere ($p<0.001$). Lipid peroxidation was elevated in whole brain ($p<0.001$), cerebral hemisphere ($p<0.02$), cerebellum ($p<0.001$) and in brain stem ($p<0.02$). In spite of the varied response of cholesterol and phospholipids levels, the cholesterol/phospholipids ratio increased in all brain regions.

*Statistically significant

Author's Conclusions:

"In this present study, the activity of lipase had also shown elevation in the cerebral hemisphere. On the basis of this report, it can be assumed that the increase in lipase activity in the cerebral hemisphere might be responsible for the decrement of total lipids in different regions of the rat brain. Further, studies utilizing radioactive isotope labeling might allow a better insight into the effect of SO_2 on the rate of turnover of lipid fractions in the different areas of the brain."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* +b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation ☐ *Guideline: Below 20% variation* +f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? +✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.*

	+✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* +✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+/-✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +/-✏ Was a full description of the monitoring devices provided? +/-✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✏ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? ±✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? +✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✏ Was individual data reported for each test animal?
G. Data analysis:	+✏ Are the statistical methods used appropriate? ±✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective?

	+✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results of this study are novel in that they provide neurochemical information on regional lipid changes in the rat brain. This study suggests that SO_2-exposure induces degradation of lipids. In the future, studies utilizing radioactive isotope labeling might allow a better insight into the effect of SO_2 on the rate of turnover of lipid fractions in the different areas of the brain.

Confidence index:

Sufficient sample size to identify statistically significant changes. Methods used in this study did not monitor the response of rats to SO_2 exposure while they were alive, rather, consideration was only given to the rats after death. In addition, no mention was made of rats' age or pre-trial health. No gradient of exposure was used, and only nominal exposure levels were recorded. Dimensions and other description of the exposure chamber was mentioned in a previous paper. However, it is unclear if animals were acclimated prior to the experiment. Results are in agreement with studies done in guinea pigs showing that the similar exposure regimen produced a significant depletion of total lipids. Exposure conditions and assessment methods are well defined – study should be easily reproducible. Use of radioactive isotope labeling would provide better insight into effect of SO_2 on lipid turnover in different areas of the brain – previous studies have shown that SO_2 can accumulate in the brain. Used dose of SO_2 that is equivalent to maximum allowable concentration. Use of same sex, age and approx. weight of animals limit confounding factors.

Review – Confidence index ranking:

Moderate to high

Author: Knauss, HJ., Robinson, WE., Medici, TC. and Chodosh, S. **Internal ID:** 250

Title: Cell vs. Noncell Airway Temporal Responses in Rats Exposed to Sulfur Dioxide

Year: 1976

Abstract: A modification of the sulfur dioxide exposed rat model proposed by Reid for the study of chronic bronchitis was employed to evaluate mucus retention and cytologic changes. Rats were exposed to from 600 to 700 ppm of SO_2 for 3 hours per day, and groups were examined after 0, 9, 18 and 30 hours of cumulative exposure. Tracheal mucus retention and statistically significant increases in the amount of solid material recovered by bronchial lavage developed as a function of exposure time. The increase in bronchial solids was primarily due to inflammatory cells. The goblet cell population in secondary bronchi declined initially, with a significant increase after 30 hours of exposure. The cytologic data provide additional support to Reid's suggestion that the SO_2-exposed rat may be considered as a chronic bronchitis model.

Objective: The present studies were undertaken to determine the contribution of the cellular as well as the noncellular constituents to the relatively early (9-30hours postexposure) bronchopulmonary response in SO_2 exposed rats. The currently held concept that simple mucus hypersecretion is the earliest change in irritant-stimulated chronic bronchitis needs to be reassessed on the basis of the results obtained.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
600 to 700 ppm	3 hours/day x 9, 18 or 30 hours	rats	Wistar	adult	female	259	unknown	unknown	unknown

Observations:

Recovery of Cells in Lung Washes and Tracheal Mucus

Exposure of rats to SO_2 markedly increases the number of cells recovered in the bronchial washes – small but significant increases ($p<0.05$) are seen after only 9 hours of exposure. These increases in cell recovery (bronchial and tracheal) are observed to be dependent upon exposure time.

Recovery of Total Solids

The volume of total solids recovered from the bronchi increases significantly with time of SO_2 exposure

Tracheal mucus appears in significant ($p<0.05$) amounts after 9 hours of exposure, and the volume recovered, as well as the proportion of animas with visible mucus, increases with duration of exposure.

Bronchial and Tracheal Solids

Statistical comparison of the exposed and nonexposed animals during the 9- to 30-hour periods indicates no significant difference between the two groups ($p<.05$). Similar compariosns revealed that the cellular component of the bronchial solids increases significantly during SO_2 exposure ($p<.01$). The amount of tracheal secretion is significantly increased after both 18 hours exposure ($p<.05$) and 30 hours postexposure ($p<.001$) over the 9-hour level.

Goblet Cell Count

Initially, SO_2 exposure decreases the number of goblet cells in the primary and secondary bronchi-in the former the number is significantly lower ($p<.001$) after 9 hours exposure; The number of goblet cells in the secondary bronchi rises markedly and is significantly elevated ($p<.0005$) after 18 hours exposure. After 30 hours, the increase becomes approximately tenfold.

Author's Conclusions:

"The cytologic data provide additional support to Reid's suggestion that the SO_2-exposed rat may be considered as a chronic bronchitis model.."

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.*

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C. Housing/Feeding:	(-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+) Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* (+) Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* (+) Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* (+) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate?

	(+/-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+/-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results from this study indicated that tracheal mucus retention and statistically significant increases in the amount of solid material recovered by bronchial lavage developed as a function of exposure time. The findings in this study may provide information into the developmental stages of chronic bronchitis that might correspond to the preclinical condition in patents. However, the concentrations used were much higher than humans would be exposed to and comparative studies would be difficult to obtain at the human level. Also, as stated by the authors, the distribution of mucus glands in humans is different than in rats. The bacteriology and pathology of the airways of the rats exposed to SO_2 for 30 hours is not reported in this paper. Other studies have suggested that the responses noted in the experiment may not be sustained in the majority of animals if SO_2 exposure is stopped. Future studies might examine the time course of recovery to determine when the response becomes negligible.

Confidence index:

Many characteristics of the animals used during this experiment were not supplied such as age of animals and pre-trial health. Other details such as conditions within the exposure chamber were not mentioned, and it was not clear whether animals were acclimatized and whether they had been in the chamber as conditions were equilibrated. In addition, the type of food used was not characterized.

This study provided a sufficient sample size to identify statistically significant changes. Exposure conditions and assessment methods were well defined – the study would be easily reproducible.

Review – Confidence index ranking:

Moderate

Author: Langley-Evans SC. Phillips GJ. Jackson AA.

Internal ID: 251

Title: Sulphur dioxide: a potent glutathione depleting agent.

Year: 1996

Abstract: Sulphur dioxide (SO_2) is an air pollutant implicated in the initiation of asthmatic symptoms. Glutathione (GSH) has been proposed to play a role in detoxification of SO_2 through the sulfitolysis of glutathione disulphide (GSSG) to S-sulphoglutathione ($GSSO_3^-$). Rats were exposed to concentrations of SO_2 between 5 and 100 ppm for 5 hr a day between 7 and 28 days. Lung injury as assessed by bronchoalveolar lavage and tissue GSH status were evaluated. SO_2 5 ppm failed to elicit any lung injury or inflammatory response but did deplete GSH pools in lung, liver, heart and kidney. Activities of gamma-glutamylcysteine synthetase (GCS), glutathione peroxidase (GPx), glutathione S-transferase (GST) and glutathione reductase (GRed) in lung were lowered relative to those in control animals. In liver, GRed activity was decreased. SO_2 50 ppm exposure also failed to elicit injury or inflammation but did lower inflammatory cell numbers in the circulation. Rats exposed to 50 ppm SO_2 maintained tissue GSH status, but activities of GCS, GPx, GRed and gamma-glutamyltranspeptidase in lung and hepatic GRed and GPx were significantly lower than in control rats. Unaltered GST activity in lung and liver was suggestive of an impairment of the sulfitolysis reaction in these animals, perhaps through lower substrate flux through the GPx reaction, as GSSO3- is a known inhibitor of GST in the rat. Rats exposed to 100 ppm SO_2 exhibited evidence of inflammation (120-fold increase in neutrophil numbers recovered in lavage fluid) and like the 5 ppm exposed rats had lower tissue GSH concentrations and GSH-related enzyme activities in lung. We conclude that sulfitolysis of GSSG does occur in vivo during SO_2 exposure and that SO_2, even in the absence of pulmonary injury, is a potent glutathione depleting agent.

Objective: "In this article, we sought evidence of SO_2-induced changes to GSH concentrations and GSH related enzyme activities in tissues of rats exposed to varying levels of the gas. A low concentration of 5ppm was used to simulate high urban or industrial scenarios, whereas 50 and 100ppm SO_2 exposures were used to examine the extreme effects of the gas. Exposure periods of between 7 and 28 days were used to determine any temporal pattern of SO_2 related lung injury."

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
5-100ppm	**5h/day for 7-28 days**	**Rat**	**Wistar**	**7week**	**male**	**56**	**n/a**	**n/a**	**5ppm**

Observations:

100ppm SO2 caused a fourfold increase in leucocyte numbers while 5 and 50ppm did not have a significant effect.
Non dose-response changes in numbers of neutrophils were noticed following exposures to 5, 50 and 100ppm SO_2
Exposure to SO2 tended to increase blood GSH concentrations, even though the increase was non-significant in most groups
Tissue GSH concentrations were generally depleted by SO_2 exposure. Liver GSH showed major GSH depletions in response to 100ppm SO_2, while heart GSH showed patterns of decrease, resolution of injury, then decrease again. Kidney GSH status was less affected.
Activity of the synthetic enzyme, GCS, was significantly decreased by exposure to 5 or 100ppm SO_2, while 50ppm showed a much more prolonged and severe reduction.
Activity of the glutathione transport enzyme (GT) in the lungs was unaltered by 5 or 100ppm SO_2, but was significantly decreased following 50ppm exposures.
Lung GPx activity was reduced by 5 or 50ppm SO_2 exposures, but not by 100ppm.

Lung GST activity was significantly reduced by exposure to 5ppm SO_2, unaffected by 50ppm SO_2, and elevated by 100ppm SO_2. In the liver, GST activity was reduced by exposures to 5 and 100ppm SO_2, but increased by 50ppm SO_2.
*Statistically significant

Author's Conclusions:

"In summary, chronic exposures to sulfur dioxide concentrations between 5 and 100ppm failed to produce significant chronic lung injury in rats. Transient inflammatory responses were noted in conjunction with pronounced depletion of tissue GSH. The effects of sulfur dioxide did not follow a linear dose-response curve, and rats exposed to 50ppm SO_2 were able to regulate enzyme activities such that GSH status was maintained. This appeared to provide some protection from pulmonary inflammation. The basis of the sensing system for this adaptation to sulfur dioxide remains undefined."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* +b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation ☐ *Guideline: Below 20% variation* +f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +/-✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate?

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D. Equipment:	+✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✏ Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* ±✏ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? ±✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams?

	+✏ Was the timing and frequency of assessment logical? +✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✏ Was individual data reported for each test animal?
G. Data analysis:	+✏ Are the statistical methods used appropriate? -✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed? +✏ Are the results novel? +✏ Was the design of the study appropriate to investigate the primary objective? +✏ Were assumptions reasonable? +✏ Are the methods reproducible? +✏ Did either the study design or the statistical analysis limit confounding variables? +✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings demonstrate that SO_2 can act to deplete glutathione levels within the bodies of exposed rats. Prior to this study, the tripeptide glutathione was believed to be a major element in the detoxificiation of SO_2. However, results from this study indicate that high levels of SO_2 is a potent glutathione depleting agent. Consequently, an individual that undergoes chronic exposure to SO_2 may be more susceptible to the effects of toxins generated by phase one drug metabolism that are dependent on the hepatic glutathione system for detoxification. Interestingly, patterns observed do not conform to typical dose-response curves. Though clinical symptoms may not be apparent, SO_2 exposure has been shown to seriously affect production and function of glutathione in the body. More research will be needed to examine the puzzling lack of a dose-response relationship.

Confidence index:

Used a range of exposures to simulate urban, industrial or extreme effects of SO_2. Sufficient sample size to identify statistically significant changes. Exposure conditions and assessment methods were well defined – study should be easily reproducible. Used same age and sex rats to limit confounding factors. Unclear why response to SO_2 did not follow a linear dose-response curve – suggest that 50 ppm elicits an adaptive response but there is nothing to support this hypothesis. In fact, all doses caused decreases in inflammatory cell numbers and these effects were statistically significant for 50 ppm. Also there was no evidence of bronchoconstriction, airway mucous production or increases in BALF protein, which contradicts multiple published works. No evidence to warrant extrapolation to human responses.

Review – Confidence index ranking:

Moderate

Author: Husain, MM. and Dehnen, W.

Internal ID: 252

Title: Effect of NO_2 and SO_2 Inhalation on Benzo(a)pyrene Metabolism in Rat Lung

Year: 1978

Abstract: Male Wistar rats were continuously exposed to NO_2 (14.4 ppm), SO_2 (46.5 ppm) and to a mixture of both gases and their effect on lung microsomal aryl hydrocarbon hydroxylase (AHH) activity was determined. The pre-exposed animals were administered methylcholanthrene (MC) to investigate the exposure effect on enzyme inducibility and pattern of B(a)P metabolites. NO_2 significantly increased AHH activity but no marked change was noted with SO_2. Induction of AHH by MC was markedly inhibited by SO_2, only slightly by mixture of NO_2-SO_2 but not with NO_2 alone.

Objective: To determine the effect of NO_2 and SO_2 inhalation on benzo(a)pyrene metabolism in rat lung.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
46.5 ppm	Continuously for up to 4 weeks	rats	Wistar	adult	male	60	unknown	unknown	unknown

Observations:

After 2 weeks exposure, the weight of the exposed animals was significantly lower than of the other groups. This difference wasn't present at 4 weeks.

Exposure to SO_2 (46.5ppm) did not induce any appreciable change compared to the control groups.

The exposure to the mixture of both gases did not alter the rate of B(a)P-hydroxylation.

Inhalation of SO_2 markedly ($p=0.001$) reduced (49%) enzyme induction compared with unexposed rats. Rats exposed to the mixture of these gases also showed a slight reduction of enzyme induction which was significant only at the 5% level.

*Statistically significant

Author's Conclusions:

"It could not be ascertained from the present studies that the observed effects after NO_2 exposure were due to alterations in the enzyme itself. The increase in enzyme activity perhaps may be to some extent related to the cellular damage, appearance of new types of cells or due to the formation of lipid peroxides in rat lung. In view of the possibility that some elements and compounds may act as cofactors in carcinogenesis by inhibiting or activating enzymes that detoxify inhaled carcinogens that observations even with the high concentration of gases used may have some significance. Since sufficient research has not been done in this aspect further investigations are needed."

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (+) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+/-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective?

	(-) Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
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	nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+/-) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+/-) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Few studies are available to examine the interactions between pollutant gases and PAH metabolizing enzymes. This study examined the effect of a common pollutant, SO_2, on benzo(a)pyrene metabolism. Results indicated that SO_2 had no marked effect on benzo(a)pyrene metabolism. Although the dose of SO_2 was very high, the results may have some implications in terms of the ability of SO_2 to enhance the carcinogenic potential of polyaromatic hydrocarbons that may be metabolized by AHH. Insufficient data to warrant extrapolations to human responses.

Future studies on the effect of other common pollutants on the inhibition or activation of enzymes that may detoxify inhaled carcinogens are worthwhile.

Confidence index:

This study used a sufficient sample size for identifying statistically significant changes. Furthermore, a description of the exposure chamber was given along with climatic conditions maintained throughout the study. However, pre-trail conditions were not given, and it was not clear if rats were exposed to SO_2 during equilibration. Nonetheless, the study did describe many of the characteristics of the animals involved in the study such as gender, strain and species. However, the age of the rats was not given, a characteristic that could confound results if not stated.

Review – Confidence index ranking:

Moderate to low

Author: Leong KJ, MacFarland HN

Internal ID: 253

Title: Pulmonary dynamics and retention of toxic gases: I. Sulfur dioxide: Concentration and duration effects in rats.

Year: 1965

Abstract: n/a

Objective: "This paper presents a method which combines a respirographic technique with an analytical method and furnishes results obtained in rats undergoing an inhalation exposure to sulfur dioxide. The effects of changes in concentration of the gas and in duration of exposure on pulmonary retention, tidal volume, and respiratory rate are described."

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
40-750ppm	**2hrs**	**Rat**	**Wistar**	**n/a 190-210g**	**n/a**	**n=10 x 7**	**n/a**	**n/a**	**40ppm**

Observations:

Almost all animals demonstrated respiratory stress following exposure to SO_2

"In general, percent retention (SO_2), respiratory rate, and minute volume varied inversely as the concentration of sulfur dioxide, whereas tidal volume varied directly. On the other hand, the duration of an exposure affected mainly the percent retention, and to some extent, the respiratory rate."

"The most striking microscopic finding was the positive correlation between the frequency of occurrence of pulmonary damage and the concentration of sulfur dioxide. For example, 70 to 80% of the lungs from rats exposed respectively to 426 to 751 ppm of the gas showed various degrees of pulmonary edema, whereas only 10 to 30% of the specimens from groups of animals subjected to low levels (60 to 200ppm) of the agent demonstrated similar types of the lesion. On the other hand, none of the lungs of the animals from 40ppm and the control groups had observable adverse histological changes."

*Statistically significant

Author's Conclusions:

"The results of the biological studies described above indicated that the concentrations of sulfur dioxide in the inspired air influenced to various degrees the percent retention of the gas by the respiratory system and also affected pulmonary dynamics."

The study suggested "…the existence of two distinct phases or methods in the pulmonary system which mediate the alteration of percent retention with respect to the sulfur dioxide concentration in the inspired air. The first mechanism, operative only at low sulfur dioxide levels may be described as a fast, physiological response… it is reasonable to suggest that the initial rapid decrease in α (percent retention) is a result of a rapid change in the respiratory tract such as bronchoconstriction, and hence, the absorptive conditions. The second mechanism, operative at high concentrations of sulfur dioxide, may be ascribed to a slow pathological response superimposed on the maximum physiological response. As accumulation of bronchial secretions and development of pulmonary edema progress, the absorptive processes of gas may be interfered with to an increasing extent."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? +✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +/-✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +/-✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.*

	-✐ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✐ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+✐ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✐ Was a full description of the monitoring devices provided? -✐ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✐ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✐ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✐ Were test animals randomly assigned to exposure groups? -✐ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✐ Was a control group assayed? +✐ Is the control regime appropriate? +✐ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✐ Were appropriate endpoints examined? +✐ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✐ Were accepted assessment methods employed? +✐ Were assessment measures sufficiently sensitive? +✐ Was the nature and severity of symptoms graded against an explicitly defined scale? +✐ Did qualified personnel conduct the exams? +✐ Was the timing and frequency of assessment logical? +✐ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✐ Was individual data reported for each test animal?
G. Data analysis:	+✐ Are the statistical methods used appropriate? -✐ Are confidence intervals reported? +✐ Did the investigator appropriately interpret the statistics? +✐ Are the observed trends statistically significant?
H. Interpretations:	+✐ Was the original objective addressed? +✐ Are the results novel? +✐ Was the design of the study appropriate to investigate the primary objective?

	+✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? ±✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study presents a method that combines a respirographic technique with an analytical method and provides results obtained in rats undergoing an inhalation exposure to sulfur dioxide. The study focuses on the pulmonary retention system and essentially uses the experiment as an example. At the time, it provided a novel approach to describe the events that occurred when an animal was exposed to an airborne toxicant. The data suggest that two distinct phases exist in the pulmonary systems, which mediate the alteration of percent retention with respect to SO_2 concentration in the inspired air. This has implications with regards to even simple extrapolations from the data, which do not follow a linear dose-response curve. No evidence that similar pulmonary dynamics occur in humans.

Confidence index:

It focuses on a system of studying pulmonary retention and does not focus as much on the characteristics of animals used in the study. Age, sex and pre-trial health are not mentioned. Pre-trial conditions, acclimation times and type and amount of feed were also omitted. The study also does not describe the climactic conditions in the exposure chamber, but refers readers to a separate paper for those details. Sufficient sample size to identify statistically significant changes. Novel method to assess pulmonary retention of SO_2. Also assessed multiple parameters for lung function and performed histological analysis. Exposure conditions and assessment methods were well defined- study should be easily reproducible. Used same weight rats, and each rat served as its own internal control to limit confounding factors.

Review – Confidence index ranking:

Moderate to high

Author: Lee, SD. and Danner, RM. **Internal ID:** 254

Title: Biological Effects of SO_2 Exposures on Guinea Pigs.

Year: 1966

Abstract: Although considerable data concerning the toxicological and physiological effects of SO_2 exposure on humans and experimental animals are available in the literature, little information concerning the biochemical effects of SO_2 exposure on experimental subjects is available. The current investigations were initiated to provide a basis for a more critical evaluation and interpretation of studies related to SO_2. This preliminary communication concerns observations made in a series of experiments conducted to establish the minimum SO_2 exposure concentration producing consistent detectable alterations in the respiration and biochemistry of experimental animals. Hemoglobin and blood inorganic sulfur concentrations were determined to detect alterations occurring in the blood compartment, which serves as the vehicle for translocation of absorbed sulfur.

Objective: To establish the minimum SO_2 exposure concentration producing consistent detectable alterations in the respiration and biochemistry of experimental animals.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
6 to 310 ppm	60 min	Guinea Pigs	unknown	1 to 1.5years	unknown	36	unknown	unknown	unknown

Observations:

SO_2 exposures at concentrations above 19 ppm for one hour caused an increase in tidal volume and a decrease in respiration rate, with irregular effects on minute volume. SO_2 exposures at concentrations ranging from 7 to 17 ppm caused a general decrease in tidal volume and an increase in respiration rate. Hemoglobin concentrations increased after SO_2 exposure with all concentrations tested. Control animals showed decreased hemoglobin concentrations following a comparable test period without SO_2 exposure. Blood inorganic sulfur concentrations increased after exposures to SO_2 concentrations above 19 ppm and exhibited irregular responses below 17 ppm of SO_2. Control animals showed decreased blood inorganic sulfur concentrations.

Author's Conclusions:

"These results are in general agreement with those reported for guinea pigs and human subjects receiving exposures of 15 to 30 ppm SO_2. Further investigations are being conducted to elucidate the mechanism responsible for the changes observed during these preliminary experiments."

Review – Assessment:

A. Test animals:	(-) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+/-) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.*

	(+) b) Age ↳ *Guideline: Adult, i.e.,8-12 weeks.* (+/-) c) Sex (+/-) d) Pre-test health ↳ *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation ↳ *Guideline: Below 20% variation* (+/-) f) Source ↳ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? ↳ *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? ↳ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ↳ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) Was the number of animals grouped in a single chamber stated? ↳ *Guideline: Groupings should permit clear observation of each animal.*

	(+) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+/-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+/-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions?

	(+) Was individual data reported for each test animal?
G. Data analysis:	N/A Are the statistical methods used appropriate? N/A Are confidence intervals reported? N/A Did the investigator appropriately interpret the statistics? N/A Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+). Was the design of the study appropriate to investigate the primary objective? (+/-) Were assumptions reasonable? (+) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The experiment was done to establish the minimum SO_2 exposure concentration that would consistently affect the respiration and biochemistry of guinea pigs. This study identifies 19 ppm as the minimum SO_2 exposure level that produced consistent detectable alterations in respiration and hemoglobin and blood inorganic sulfur concentrations. Suggests that these criteria may aid in the evaluation and interpretation of studies related to SO_2. Results are in general agreement with those reported for guinea pigs and human subjects receiving exposures of 15 to 30 ppm SO_2.

Confidence index:

This study fails to mention the strain, sex and pre-trial health of guinea pigs involved in the study, factors that could confound the study if not accounted for. Pre-testing conditions such as feed and feeding schedule are omitted, as are most of the exposure chamber conditions. Furthermore, confidence intervals were not mentioned and the investigators did not account for both nominal and actual exposure levels. Results were not analyzed using statistics. Furthermore, each animal served as its own control but there was only a single animal for each dose. The respiration data was in good agreement with those reported for both guinea pigs and human subjects. In addition, the exposure conditions were well defined, but assessment methods had no details. Consequently, the study would not be easily reproducible.

Review – Confidence index ranking:

Low

Author: Mannix, RC., Phalen, RF., Walters, RB. and Kurosaki, TT. **Internal ID:** 256

Title: Effects of Sulfur Dioxide and Formaldehyde on Particle Clearance in the Rat

Year: 1983

Abstract: The effects of exposures to sulfur dioxide and formaldehyde atmospheres on the clearance of inhaled, insoluble tracer particles from the lungs of rats have been studied. The tracer particles employed were polystyrene latex microspheres radiolabeled with ^{51}Cr. Following the deposition of the 1.9 um activity median aerodynamic diameter particles, the rats were divided into 3 groups for a single 4-hour exposure to purified air, 20 ppm sulfur dioxide, or 20 ppm formaldehyde. Early, presumably upper-respiratory-tract, clearance was monitored by analysis of radioactivity excreted in feces, while late, presumably deep-lung, clearance was followed by thoracic counting of the animals. Both the sulfur dioxide and formaldehyde atmospheres did significantly delay early clearance. However, the late clearance rates of the two pollutant-exposed groups of rats were not significantly different from that of the purified air-exposed group of rats. Although sulfur dioxide had numerically greater effects than formaldehyde, the differences were not statistically significant at the $p<0.1$ level.

Objective: To demonstrate the effects of sulfur dioxide and formaldehyde on particle clearance in the rat.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
20 ppm	4-hour	rat	Sprague-Dawley	unknown	male	unknown	unknown	unknown	unknown

Observations:

Both the sulfur dioxide and formaldehyde atmospheres did significantly delay early clearance. However, the late clearance rates of the two pollutant-exposed groups of rats were not significantly different from that of the purified air-exposed group of rats. Although sulfur dioxide had numerically greater effects than formaldehyde, the differences were not statistically significant at the $p<0.1$ level.

*Statistically significant

Author's Conclusions:

"The purpose of this study was to determine the similarities and differences between the effects of SO_2 and HCHO using particle clearance endpoints. Both of these gases are highly soluble in the mucus lining the upper airways, and under normal conditions they would not be expected to penetrate in significant amounts to the deep lung. Therefore, one might suspect that clearance in the upper respiratory tract could be affected by exposure to these gases, but that late clearance phenomena (macrophage engulfment, solubilization, penetration through tissues), associated with the deep lung (respiratory bronchioles and more distal structures), would be unaffected. The results of the study seem to support the above hypotheses. Early, presumably upper-respiratory-tract, clearance was significantly delayed by 20-ppm levels of SO_2 and HCHO, to roughly the same extent. Both of the endpoints used in characterizing late clearance were in agreement-neither the SO_2 nor HCHO significantly affected late, presumably deep-lung, clearance. Therefore, it appears that SO_2 and HCHO at the concentration studied have nearly identical effects upon particle clearance in the rat."

Review – Assessment:

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex (+) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (+) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(+) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary

	objective? (-) Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+/-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(+) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (+/-) Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough (*i.e.,* information pertaining to the clinical

	nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+). Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results of the study support the hypothesis that SO_2 wouldn't be expected to penetrate in significant amounts to the deep lung. As expected, the SO_2 did not significantly affect late, deep-lung clearance. This study further indicated that SO_2 and HCHO have nearly identical effects on particle clearance in the rat. This concentration of SO_2 used in the study far exceeds the Federal PEL, also, nothing in the study warrants extrapolation to human responses as there was no comparison of the lung clearance kinetics for humans and rats.

Confidence index:

Results from this study left out a number of significant factors including age and pre-trial health. Furthermore, whether animals were in the chamber during equilibration was not clear. No gradient of exposure levels was used and only nominal SO_2 levels were recorded. Finally, amount and feeding schedule was not specified. However, the study used a sufficient sample size to identify statistically significant changes. The exposure conditions and assessment method were well defined. Consequently, the study should be easily reproducible.

Review – Confidence index ranking:

Moderate to low

Author: McJilton CE. Frank R. Charlson RJ.	**Internal ID: 257**
Title: Influence of relative humidity on functional effects of an inhaled SO_2-aerosol mixture.	
Year: 1976	

Abstract: Lightly anesthetized guinea pigs were exposed to 1 ppm of sulfur dioxide (SO_2) and 1 mg per m^3 of sodium chloride aerosol, individually and in combination, at low and high relative humidities. At low relative humidity (less than 40 per cent) the aerosol was a crystal, at high relative humidity (greater than 80 per cent) a droplet. Exposures lasted one hour. Changes in pulmonary mechanical function characterized by an increase in flow resistance and decrease in compliance were seen only when the mixture was administered at high relative humidity. The effect is ascribed to absorption of the highly soluble SO_2 into the droplet before inhalation.

Objective: "We hypothesized that an interaction would occur between SO_2 and the NaCl aerosol if the latter were an aqueous droplet (high RH), but not if it were a dry crystal (low RH). As a droplet, the aerosol would absorb SO_2 to produce an acid solution. Synergism might then be expected for two reasons: The aerosol acted as a carrier to increase the amount of SO_2 delivered beyond the upper airways; the low pH of the droplet was a direct cause of irritation. To test this hypothesis, we manipulated the RH of the chamber containing the gas and the aerosol."

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
1ppm SO_2 and/or 1mg/m^3	**Two 60min exposures**	**Guinea pig**	**n/a**	**n/a 334±31g**	**n/a**	**36**	**n/a**	**n/a**	**1ppm SO_2 and 1mg/m^3 + 80% RH**

Observations:

The pulmonary resistance increased significantly only when the SO_2 aerosol mixture was administered at high relative humidity.* This increase in pulmonary resistance occurred within the first 15 min of exposure and was sustained during the second half hour. Dynamic pulmonary compliance decreased as pulmonary resistance increased during administration of the SO_2 aerosol mixture.* Neither tidal volume nor end-expiratory pressure was affected by exposure. The wet/dry lung weight ration was also increased in this treatment group of animals versus controls. The average pH of the condensate collected from the gas-aerosol mixture at high relative humidity was 3.2 versus 6.8 for aerosol alone. There was no significant change in pulmonary flow resistance with SO_2 exposure only.

*Statistically significant

Author's Conclusions:

"The only circumstance in which significant changes in respiratory mechanical function were elicited occurred when 1ppm and 1mg per m^3 were administered together at high RH (relative humidity). Administration of either the gas or the aerosol alone or of the combination at a low RH was ineffectual. We suggest that this "synergism" was related to the conversion of the dry crystalline NaCl to a droplet, accompanied by the absorption of SO_2… The absorption of SO_2 by saline (or water) leads to the formation of sulfurous acid and a lowering of pH. We do not know to what extent the changes in respiratory mechanics were related to the acidity of the aerosol, to the HSO_3^- that was demonstrated, to the sulfite ion SO_3^{2-} that theoretically was present, or to the transport of SO_2 by the aerosol to lower

airways in amounts greater than would have occurred if the gas alone were administered."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* -✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.*

	±✏ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* -✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✏ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+✏ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✏ Was a full description of the monitoring devices provided? -✏ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✏ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✏ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✏ Were test animals randomly assigned to exposure groups? -✏ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✏ Was a control group assayed? +✏ Is the control regime appropriate? -✏ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✏ Were appropriate endpoints examined? +✏ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✏ Were accepted assessment methods employed? +✏ Were assessment measures sufficiently sensitive? +✏ Was the nature and severity of symptoms graded against an explicitly defined scale? +✏ Did qualified personnel conduct the exams? +✏ Was the timing and frequency of assessment logical? +✏ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✏ Was individual data reported for each test animal?
G. Data analysis:	+✏ Are the statistical methods used appropriate? +✏ Are confidence intervals reported? +✏ Did the investigator appropriately interpret the statistics? +✏ Are the observed trends statistically significant?
H. Interpretations:	+✏ Was the original objective addressed?

	+✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings demonstrate that low levels of SO_2 (1ppm) or NaCl (1mg/m^3) do not have an effect on pulmonary mechanics alone, but that in conjunction, and when exposed under conditions of high relative humidity, they can combine to produce significant effects. The authors propose several mechanisms by which this can occur, and suggest that further research will be needed to fully understand the phenomenon. Such synergisms may exist in the workplace or ambient environment where multiple exposures occur concurrently.

Confidence index:

Sufficient sample size to identify statistically significant changes. Exposure conditions and assessment methods were well defined – study should be easily reproducible. Only assessed pulmonary function by measuring resistance and compliance. Should have measured the amount of SO_2 dissolved in the droplet. Each animal served as its own internal control and underwent two random exposure regimens, also had aerosol alone and SO_2 alone controls, same weight animals used, light sedation to minimally depress respiration and no experiment begun until respiratory frequency of 60 per min was reached – tried to limit confounding factors. Data are in agreement with Amdur's work.

Review – Confidence index ranking:

Moderate to high

Author: Lewis, AJ. And Kirchner, T.	**Internal ID:** 258
Title: Modulation of Sulfur Dioxide-Induced Airways Hyperresponsiveness in the Conscious Dog	
Year: 1984	
Abstract: Brief exposure of conscious dogs to 30 ppm SO_2 directly into the lungs resulted in a transient bronchoconstriction followed by a period of hypersensitivity to aerosolized methacholine and 5-hydroxytryptamine. The hypersensitivity was maximal 4 hours after exposure to SO_2. Both Wy-41, 195 and disodium cromoglycate, two antiallergic drugs, inhibited the hypersensitivity to methacholine when administered just prior to methacholine challenge. Neither drug significantly influenced SO_2-induced bronchospasm nor influenced methacholine hypersensitivity when administered just prior to SO_2 exposure. Furthermore, Wy-41, 195 was ineffective against methacholine hypersensitivity when administered orally for 4 days. SO_2-induced airway hypersensitivity in the dog may thus provide a useful model for exploring mechanisms and pharmacological modulation of bronchial hyperreactivity, a common feature of asthma.	
Objective: To determine the ability of SO_2 to produce hyperresponsiveness to methacholine and 5-hydroxytryptamine in dogs.	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
10 to 30 ppm	5 min	dogs	"mongrel"	unknown	male and female	unknown	unknown	unknown	unknown

Observations:

Exposure to 10 ppm or compressed air alone was without effect on pulmonary resistance or compliance. At 30 ppm SO_2 there was a hyper responsiveness to aerosolized methacholine and the compliance was further reduced. The hypersensitivity was maximal 4 hours after exposure to SO_2. Both Wy-41, 195 and disodium cromoglycate, two antiallergic drugs, inhibited the hypersensitivity to methacholine when administered just prior to methacholine challenge. Neither drug significantly influenced SO_2-induced bronchospasm nor influenced methacholine hypersensitivity when administered just prior to SO_2 exposure. Furthermore, Wy-41, 195 was ineffective against methacholine hypersensitivity when administered orally for 4 days.
*Statistically significant

Author's Conclusions:

"SO_2-induced airway hypersensitivity in the dog may thus provide a useful model for exploring mechanisms and pharmacological modulation of bronchial hyperreactivity, a common feature of asthma. It appears that the airway hyperresponsiveness in dogs produced by exposure to 30 ppm SO_2 is inhibited by high aerosolized concentrations of the antiallergic drugs Wy-41, 195 and DSCG."

Review – Assessment:

A. Test animals:	. (+/-) Is the number of animals sufficient to produce statistically significant results?

	Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased. Are animal characteristics adequately recorded and appropriate for the objective of the study? (-) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+/-) c) Sex (+/-) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+/-) e) Weight variation *Guideline: Below 20% variation* (+/-) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+/-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?

C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+/-) Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (+/-) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+/-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+) Was a full description of the monitoring devices provided? (-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and

	dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+). Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+/-) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (+/-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The present demonstrates that at 30 ppm SO_2 there was a hyper-responsiveness to aerosolized methacholine and compliance was further reduced. A similar hyper responsiveness was observed with aerosolized 5-HT. Drugs were not found to significantly influence SO_2-induced bronchospasm. However, since number of dogs used in the study was not explicitly stated, it is unclear whether results can be interpreted as statistically significant. Nonetheless, the author postulates that SO_2-induced airway hypersensitivity in the dog could provide a useful model for exploring mechanisms and pharmacologic modulation of bronchial hyperreactivity, a common feature of asthma.

Confidence index:

This study does not explicitly describe the number of animals used nor does it describe pre-trail health or age of the dogs. No description was given of pre-trial conditions including amount and type of feed, climatic conditions and whether animals were acclimated prior to the study. In addition, dogs were conscious during the exposures but their behavior during exposures was not recorded. Furthermore, justification was not given for the use of dogs as test animals. It is also unclear whether dogs were randomly assigned to test groups. However, data from the study are in agreement with published results using human and dog studies. Single endpoint of pulmonary resistance was used to assess bronchial hyper reactivity. Future studies should look at additional parameters such as measuring mediator release and morphological changes including mucosal inflammation. Exposure conditions and assessment were well defined – study should be easily reproducible.

Review – Confidence index ranking:

Low to Moderate

Author: Park JK. Kim YK. Lee SR. Cho SH. Min KU. Kim YY.	**Internal ID: 259**
Title: Repeated exposure to low levels of sulfur dioxide enhances the development of ovalbumin-induced asthmatic reactions in guinea pigs.	
Year: 2001	

Abstract: BACKGROUND: Sulfur dioxide (SO_2) is one of the major air pollutants. It is known to aggravate asthma symptoms in human beings, but few studies have focused on the effects of SO_2 upon the development of bronchial asthma in animal models. **OBJECTIVE:** This study was undertaken to evaluate the role of SO2 upon the development of ovalbumin (OA)-induced asthmatic reactions in guinea pigs. **METHODS:** Guinea pigs were divided into four groups: (1) OA- and SO_2-exposed group (n = 12), (2) SO_2-exposed group (n = 12), (3) OA-exposed group (n = 11), and (4) saline-exposed group (n = 7). Guinea pigs of the first and second groups were exposed to 0.1 ppm SO_2 for 5 hours a day on 5 consecutive days. Guinea pigs in the first and third groups inhaled 0.1% OA aerosols for 45 minutes a day on days 3, 4, and 5. One week after the sensitization procedure, all the guinea pigs underwent bronchial challenge with 1.0% OA aerosols, using unrestricted whole-body plethysmography. Bronchoalveolar lavage and histopathologic examination were performed 24 hours after the bronchial challenge. **RESULTS:** Increases in enhanced pause (Penh), as an index of airway obstruction, after the bronchial challenge was significantly higher in OA- and SO_2-exposed group (group 1) than the other groups ($P < .05$, respectively). Eosinophil counts in bronchoalveolar lavage fluids were also significantly higher in group 1 than in the other groups ($P < .05$, respectively). Histopathologic findings of bronchial and lung tissue in the group 1 showed an infiltration of inflammatory cells, bronchiolar epithelial damage, and mucus and cell plug in the lumen, but no significant abnormalities were observed in the other groups. **CONCLUSIONS:** These results indicate that repeated exposure to low levels of sulfur dioxide may enhance the development of ovalbumin-induced asthmatic reactions in guinea pigs.

Objective: "This study was undertaken to evaluate whether repeated exposure to low levels (0.1ppm) of SO_2 could enhance the development of OA-induced asthmatic reactions in guinea pigs. To test this hypothesis, bronchial obstruction was measured using single-chamber whole-body plethysmography, and inflammatory changes in the airways were evaluated by examining bronchoalveolar lavage (BAL) fluids and the lung tissue of guinea pigs with a sensitization to OA caused by low levels of sulfur dioxide."

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
0.1ppm	**5h/d for 5 days**	**Guinea pig**	**Dunkin-Hartley**	**n/a** **250-300g**	**Male**	**42**	**n/a**	**n/a**	**0.1ppm**

Observations:

Respiratory pause increased significantly following exposure to SO_2 and OA (57.4±10.9%), as did the severity of bronchial obstruction after 1.0% OA challenge ($p<0.05$). As well, BAL fluids from animals exposed to SO_2 and OA contained significantly higher numbers of eosinophil counts. There was also evidence of increased inflammatory reactions and epithelial damage in the same exposure group.
*Statistically significant

Author's Conclusions:

"In conclusion, this study demonstrates that low levels of SO_2 enhance the development of OA-induced asthmatic reactions in guinea pigs. The results suggest that repeated exposure to low levels of SO_2 with causative allergens may enhance the development of asthma in susceptible individuals."

Review – Assessment:

A. Test animals:	+✏ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✏ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✏ Were the chosen exposure levels appropriate to investigate the primary objective? +✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +-✏ Were both nominal and actual exposure concentrations recorded? -✏ Was the duration of exposure appropriate to investigate the primary objective? +✏ Was the duration of exposure precisely defined? +/-✏ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✏ Was the mode of administration appropriate? +✏ Was the frequency of administration appropriate to investigate the primary objective? +✏ Was evidence provided to justify the dosing regime? +✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✏ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%*

	-✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* ±✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? +/-✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? +✎ Are confidence intervals reported? +✎ Did the investigator appropriately interpret the statistics?

	+✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study demonstrates that repeated exposure to low levels of SO_2 in conjunction with ovalbumin sensitized guinea pigs to a subsequent challenge with ovalbumin as assessed by both morphological changes and airway obstruction.

Confidence index:

The design used in the study was reasonably well-detailed. Only the age and pre-trial health of guinea pigs was not recorded. Pre-trial conditions were described, although it was not clear if animals were exposed during the equilibrium period. Furthermore, it was not stated whether animals were randomly divided into groups. Sufficient sample size to identify statistically significant changes. Multiple parameters were assessed including histopathological changes and airway obstruction. Preliminary studies performed with OA to identify concentrations that would induce asthma and then used a dose that was below this level for SO_2 studies. Used levels of SO_2 that are found in urban areas, but still extrapolations to human responses in unwarranted as mechanism for induction of asthma in either species is not known. Exposure conditions and assessment methods were well defined – study should be easily reproducible. Used all male guinea pigs on an OA free diet – limit confounding factors.

Review – Confidence index ranking:

High to moderate

Author: Gokemeijer, J.D.M., de Vries, K., Orie, N.G.M.	**Internal ID:** 260
Title: Response of the bronchial tree to chemical stimuli	
Year: 1973	
Abstract The purpose of this investigation was to study the broncho-obstructive activity of some air-pollutants in patients with chronic non-specific lung diseases and the mechanism involved. Sulfur dioxide, nitrogen dioxide and ozone inhalation experiments have been performed in patients under the age of 40.	
Objective: The purpose of this investigation was to study the broncho-obstructive activity of some air-pollutants in patients with chronic non-specific lung diseases and the mechanism involved.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
10 ppm SO_2	3 minutes	No info	<40 yrs	52 (divided among experiments)	Chronic non-specific lung diseases	N/A	N/A

Observations:

SO_2 caused a marked bronchial obstruction with a maximum increase in viscous work of breathing of 460 per cent at the end of the three minutes inhalation period and a recovery in 45-60 minutes.

There was a highly significant correlation between a positive SO_2 response and the presence of blood-eosinophilia and also between a positive SO_2 response and a high titer of the skin test with house dust allergen.

Protective experiments with an anticholinergic drug produced only slight protection against the SO_2 bronchus obstruction in 5 out of 9 patients. In contrast, the beta2 receptor stimulating drug, fenoterol, caused a remarkable protection against SO_2 provocation in all patients.

*Statistically significant

Author's Conclusions:

"We conclude that sulfur dioxide causes bronchoconstriction at lower concentrations when it is inhaled in dry air than when it is inhaled in partially humidified warm air. Furthermore, at least with oral breathing, concentrations of SO_2 as low as 0.1 ppm may cause modest potentiation of the bronchoconstriction produced by hyperpnea with dry air."

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+/-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study?

	(+/-)Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? (No info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+/-) Were subjects informed about the experimental risks? (No info) Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? (+)Were the chosen exposure levels appropriate to investigate the primary objective? (+/-)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+/-)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+/-)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+/-)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+/-)Were the subjects randomly assigned to exposure groups? (-)Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+)Is the control regime appropriate? (-)Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical?

	(-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+/-)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+/-)Are the observed trends statistically significant?
G. Interpretations:	(+Was the original objective addressed? (+/-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+/-)Were assumptions reasonable? (+)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study concluded that sulfur dioxide causes bronchoconstriction at lower concentrations when it is inhaled in dry air than when it is inhaled in partially humidified warm air. Furthermore, concentrations of SO_2 as low as 0.1 ppm were seen to cause modest potentiation of the bronchoconstriction produced by hypernpnea with dry air. However, given the small sample size, it is uncertain whether results could be considered statistically significant.

Confidence index:

The subjects involved signed consent forms and were informed of the risks involved in the study. However, no mention is made of how they were recruited, or if any chose to withdraw from the study. And while randomization was incorporated into the study design, no mention is made of how the randomization was done. Finally, the small number of samples used in this study does not permit this result to be overly representative of the population involved.

Exposure conditions and assessment methods were well defined – study should be easily reproducible. Each patient served as his own internal control, however, only CNSLD patients used and protective studies only performed with subset of original group. However, the methods section contains practically no information on data collection, exposure conditions, subjects, equipment or procedures. Furthermore, the assessment measurements were not discussed in the method section.

Review – Confidence index ranking:

Moderate

Author: Johnson HD, Lincoln EM, Flatt RE **Internal ID: 261**

Title: Sulfur dioxide (SO_2) exposure and recovery effects on mice

Year: 1972

Abstract: "Continuous exposure of mature, male mice to 40ppm atmosphereic SO_2 depressed feed and water intake, body weight, and O_2 consumption; and caused upper respiratory damages. After approximately 20 days of exposure, feed and water intake returned to control values, although body weight and metabolism remained low. Immediately following cessation of SO_2 exposure, the animals began to recover body weight and overcompensated in feed and H_2O intake. Oxygen consumption was normal at 32-34 days postexposure; and limited histological evidence showed no apparent damaging effects of exposure on the surviving mice."

Objective: "…the objectives of this study were to gain a better understanding of the prolonged effects of atmospheric SO_2 on the gross pulmonary responses; nasal and pulmonary tissue changes in mice; and the ability to recover from, or compensate for such exposures."

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
40ppm	**4-11days**	**mice**	**Charles River**	**mature 200days**	**male**	**24**	**n/a**	**n/a**	**40ppm**

Observations:

Feed intake (g/day), water intake (ml/day) and body weight (g) were all significantly lower in SO_2 exposed mice during the 4-11day exposure period ($p<0.05$). Post-exposure (days 5-12 postexposure), feed intake was higher in SO_2 exposed mice ($p<0.05$), water intake was non-significantly higher, and body weight remained significantly lower ($p<0.01$). When exposed to SO_2 for a longer period (33days), the above parameters eventually recovered to control levels. Histological examination revealed abnormalities in the upper respiratory tract of mice, including stratified squamous epithelium in the tract tube 3-4 layers thick, turbinate bones were thin and disorganized, and in some cases dissolution of the turbinate bones was noticed as a result of the chronic inflammation.

*Statistically significant

Author's Conclusions:

"The conclusion, after about 20 days of SO_2 exposure, it appears that the mice reassumed a near-control level of feed intake, although the body weights and O_2 consumption were subnormal, indicating a decreased metabolic activity of the SO_2 exposed individuals. We propose to do further metabolic and endocrine studies to clarify mechanisms involved in this physiological adjustment to SO_2 exposure. The most amazing aspect of this study was the rapid recovery in body weight and other measured variables after cessation of SO_2 exposure. The upper respiratory tracts of the mice even appeared normal at 35 days postexposure. With the extremely high level (40ppm) of SO_2, it is surprising to see such rapid recoveries to such an irritating and unpleasant atmosphere as SO_2."

Review – Assessment:

A. Test animals:	+✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* +b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* ±e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	±✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +/-✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? +✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* +✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.*

	+✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* +✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* ±✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) +✎ Was a control group assayed? +✎ Is the control regime appropriate? +✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	+✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? ±✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective?

	+✎ Were assumptions reasonable? +✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The study demonstrates that mice are negatively affected in terms of behavior, growth, and respiratory parameters following exposure to high levels of SO_2. Interestingly, mice recover after approximately 20 days of exposure, and quickly recover following the cessation of exposure.

Prolonged exposure to SO_2 caused a marked decline in body weight and feed and water intake and O_2 consumption. It was accompanied by upper respiratory damage. Immediately following cessation of exposure, animals began to recover body weight and overcompensated in food and water intake. Oxygen consumption was normal and limited histological evidence showed no apparent damaging effects of exposure on the surviving mice. No extrapolations to human responses are warranted and the physiological symptoms are too vague to be of any value for exposure assessment purposes.

Confidence index:

This study described most of the characteristics associated with the mice. However, pre-trial health was not mentioned. In addition, no mention was made of pre-test conditions or whether animals were acclimated prior to the experiment. On the other hand, food type and amount were mentioned, as were exposure conditions such as relative humidity and temperature. Sufficient sample size to identify statistically significant changes. Novel study used multiple parameters to assess effects of chronic exposure to SO_2, physical, physiological and histological. Post exposure times were sufficient for mice to recover completely. Also identified amount of time required for the animals to adjust physiologically to the continuous exposure (20 days). Used same sex and age mice to limit confounding variables.

Review – Confidence index ranking:

Moderate

Author: Kahana, LM. And Aronovitch, M.	**Internal ID:** 262

Title: Effects of Sulfur Dioxide on Surface Properties of the Lung

Year: 1966

Abstract: The effects of sulfur dioxide on the surface properties of the lungs were studied in rats using volume pressure determinations during fluid and air filling of excised lungs to separate tissue and surface forces. In a single exposure study involving 10 rats that were exposed to a mean concentration of 688.8 ppm of sulfur dioxide for three hours, a reduction in surface forces was clearly demonstrated. No significant changes were observed during saline filling as compared with control values, but a marked decrease in mean transpulmonary pressures was evident during air filling. Similar results appear to have been obtained in a multiple exposure study in which 5 rats were submitted repeatedly to a mean concentration of 358.9 ppm of sulfur dioxide over a three-week interval. Changes in surface properties were, however, obscured by a number of accessory factors such as a difference in lung size between the control and exposed groups and other effects of sulfur dioxide, including airway obstruction and, possibly, pulmonary edema. The findings suggest a reduction in surface tension; it was speculated that this might have been caused by increased secretion at the alveolar surface producing an increased concentration of surfactant in the alveolar lining film.

Objective: To examine the effects of sulfur dioxide on the surface properties of the lungs.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
627 to 751 ppm	3 hours	rats	albino	young	male	10	unknown	unknown	unknown
257 to 450 ppm	9 x 4 hours (over 3 weeks)					5	unknown		

Observations:

Single Exposures

The mean volume-pressure curves for this group showed striking differences from the control curves. The entire volume pressure curve for air was shifted to the left so that mean pressures at all volume points during inflation and down to the 6 mL level during deflation were significantly less than those of the control group.

The mean air-minus-saline emptying curves for the test animals showed a displacement to the left of the control curve, significant down to the 6 mL level.

The compliance at the peak volume level, namely, the over-all compliance, was calculated and the compliance for the single exposure group was significantly higher than the control group ($p-.01$).

Multiple Exposures

The study of the effects of multiple exposure was hampered by experimental failures in half the animals that reduced the original group to five animals on which data are available. Some problems were severe bronchitis in some of the animals, one animal died after anesthesia.

Mean pressures were significantly less than those of the controls at all volume levels during inflation and down to the 8 mL level on deflation.
The air-minus-saline curve showed a significant displacement to the left down to the 8 mL level.
Mean overall compliance for the multiple exposures, was significantly higher than that of the control group ($p<0.001$).

*Statistically significant

Author's Conclusions:

"The findings suggest a reduction in surface tension; it was speculated that this might have been caused by increased secretion at the alveolar surface producing an increased concentration of surfactant in the alveolar lining film. It was further suggested that a similar mechanism, that is, a reduction in surface forces associated with an increase in lung compliance resulting from an inhaled pollutant, might be an important factor in the development of emphysema"

Review – Assessment:

A. Test animals:	(+/-) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+/-) b) Age *Guideline: Adult, i.e.,8-12 weeks.*

	(+) c) Sex (+/-) d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* (-) e) Weight variation ☐ *Guideline: Below 20% variation* (+/-) f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* (-) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+/-) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (+) Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* (+) Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.*

	(-) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (-) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+/-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+/-) Were test animals randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+/-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate?

	(-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (+/-) Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables? (+/-) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The effects of sulfur dioxide on the surface properties of the lungs were studied in rats using volume pressure determinations during fluid and air filling of excised lugs to separate tissue and surface forces. Few of the findings were statistically significant. The high exposure concentrations make it difficult to extrapolate to human populations. Previous studies have suggested that a primary change in lung tissue might be a sequelaue to airway obstruction and air trapping of chronic bronchitis. The single exposure studies demonstrate a pronounced decrease in pressures during air filling with no significant changes during fluid filling. Furthermore, a decline in surface forces seems to have occurred in the multiple exposure study. Future studies should attempt to clarify the mechanism that causes apparent decrease in surface tension.

Confidence index:

This study used a sufficient sample size to produce statistically significant changes. Both single and multiple exposures were performed. The concentration used far exceeded what has been observed, even in acute pollution disasters. For this reason, lower concentrations should have been tested. The exposure conditions and assessment method were well defined. Consequently, this study should be easily reproducible. This study did not mention some of the factors that could have confounded results from this study such as pre-trial health and exact age. Also, it was difficult to ascertain whether animals were randomly assigned to exposure groups. Furthermore, no description of pre-trial conditions was given, and it was not stated whether animals were acclimated prior to the study, or if animals were present in the exposure chamber during equilibration. In addition, results may have varied due to differences in lung size. Also, food type and amount were not mentioned. Confidence intervals were not listed and animals were not necessarily randomly assigned to test groups.

Review – Confidence index ranking:

Low

Author: Lippmann M. Albert RE. Yeates DB. Berger JM. Foster WM. Bohning DE.	**Internal ID: 263**
Title: Factors affecting tracheobronchial mucociliary transport.	
Year: 1975	
Abstract: In vivo mucus transport rates were studied in humans and donkeys by external measurement of the rate of clearance of insoluble monodisperse gamma-tagged aerosols. The influence of temperature, environmental toxicants and drugs which affect the autonomic nervous system were studied by determining the changes in clearance produced by them in individual subjects. In donkeys, increased pretest ambient temperature accelerated clearance by greater than 1.7%/ degrees C. Smoking 2-7 cigarettes reduced the duration of bronchial clearance by approximately 50% in both humans and donkeys. Donkeys were exposed to higher doses; with progressive slowing of tracheal and bronchial clearance for greater than 10 cigarettes. HCN, at concentrations up to 1230 ppm, produced only a mild transient slowing of clearance. In man, atropine slowed clearance, while the adrenergic stimulating drugs, isoproterenol and epinephrine, both accelerated it by greater than or equal to 4 times, as did isoproterenol when given subsequent to atropine. The cholinergic stimulating drug methacholine increased mucociliary transport in the donkey. Administration of a tap water aerosol for 10-15 min in humans increased bronchial clearance rates by approximately 25%.	
Objective: "This paper is concerned with: a) characteristics of normal mucociliary transport in the tracheobronchial tree, b) the effects of drugs which alter the autonomic control of mucociliary transport, and c) the effects of inhaled toxicants. It briefly coordinates the findings of the numerous studies on mucociliary clearance in donkeys and humans performed in this laboratory; examines the suitability of using the donkey as a model for mucociliary clearance, and gives an overview using these data and the data of other workers in the field."	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
53-300ppm	**30min**	**Donkey**	**n/a**	**n/a**	**n/a**	**3**	**n/a**	**300ppm**	**n/a**

Observations:

No effect was observed on mean residence times (MRT) following exposures of 53-300ppm. "One test performed at a lower concentration produced an MRT below test control values, suggesting that low-level exposures might accelerate bronchial clearance, as was observed for low-level cigarette smoke exposure." A similar study was performed on humans. For one subject, an exposure of 13ppm for 6 to 7min increased MRT by 30%.
*Statistically significant

Author's Conclusions:

No suitable quotations were available, as the SO_2 exposure study comprised but a small fraction of the overall work. No effects on MRT were seen in donkeys following a 30min exposure to 300ppm SO_2.

Review – Assessment:

A. Test animals:	±✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* ✎ Are animal characteristics adequately recorded and appropriate for the objective of the study? +a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* -b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* -c) Sex -d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* -e) Weight variation ☐ *Guideline: Below 20% variation* -f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	-✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? +✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? ±✎ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? -✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✎ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* +✎ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* +✎ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.*

	-✎ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* -✎ Was the amount and feeding schedule specified? ☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* -✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	-✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* -✎ Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? -✎ Were test animals randomly assigned to exposure groups? -✎ Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? -✎ Is the control regime appropriate? -✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	±✎ Were appropriate endpoints examined? -✎ Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	-✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? ±✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
H. Interpretations:	±✎ Was the original objective addressed? ±✎ Are the results novel? ±✎ Was the design of the study appropriate to investigate the primary objective?

	+✎ Were assumptions reasonable? ±✎ Are the methods reproducible? -✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings showed that donkeys exposed to 53-300ppm SO_2 for half an hour did not have differential MRT as opposed to those unexposed. A suggestion was made that lower exposure levels may speed bronchial clearance, as was witnessed in a single test. Previous work in donkeys had suggested that low-level exposures might accelerate bronchial clearance. There was a 30% increase in retention when SO_2 was inhaled prior to tagged particle inhalation. In contrast, there was no effect on bronchial clearance when inhaled after particle inhalation.

Confidence index:

The results of this "study" are provided in a single short paragraph at the end of a much larger paper. Insufficient subjects for identifying statistically significant changes. Preliminary study used a single dose, 13 ppm, which is considered low – maybe for content in cigarette smoke but this was not mentioned. Assumption was extrapolated from data in donkeys – not a good model. Exposure conditions and assessment methods were not well defined – study would not be reproducible. Data only shown for single patient. Unknown age, sex, weight – did not limit confounding factors. Used healthy subjects for extrapolation to cigarette smoke exposure. While donkeys were acclimated, the 6-hour period is not considered to be of sufficient length. Exposure chamber was defined only as an "unheated shed" and climatic conditions were not controlled.

Review – Confidence index ranking:

Low

Author: Bedi, J.F., Horvath, S.M. | **Internal ID:** 266

Title: Inhalation Route Effects on Exposure to 2.0 Parts Per Million Sulfur Dioxide in Normal Subjects

Year: 1989

Abstract: A number of investigations have attributed the control of the nasal to oral/nasal ventilation transition to nasal resistance. To investigate possible changes in nasal resistance due to sulfur dioxide (SO_2) exposure, 14 subjects (7 men and 7 women), healthy non-smokers, between the ages of 20 and 46 years, were exposed for 30 minutes to filtered air while free breathing and to 2.0 ppm SO_2 with either free breathing, forced oral or forced nasal breathing with continuous exercise at a workload 300 kg.m/min below the workload which initiated cross-over from nasal to oral/nasal breathing in a preliminary incremental workload test. An incremental work test under the ambient conditions was performed immediately following the 30-minute exercise to ascertain any change in the cross-over ventilation. Pre- and post-measures of pulmonary functions were obtained to ascertain any changes in these parameters due to the exposure. There was a significant difference in the workload at which cross-over occurred following forced oral breathing in 2.0 ppm sulfur dioxide. The nasal ventilation prior to cross-over and the nasal component of ventilation were significantly smaller for this exposure condition, indicating a possible change in nasal dynamics following the 30 minutes of forced oral breathing in 2.0 ppm SO_2. Lack of concomitant changes in pulmonary function tests including airway resistance suggests that breathing 2.0 ppm SO_2 does not affect normal subjects whether administration is by free, forced oral or forced nasal breathing.

Objective: We investigated possible changes in the respiratory pattern in normal subjects during incremental work up to cross-over breathing following a half hour of continuous exercise in 2.0ppm SO_2 with either unencumbered breathing or with forced oral or forced nasal breathing to ascertain possible changes in nasal resistance.

Overall study design:

Exposure level	**Exposure frequency/ duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
2.0 ppm SO_2	30 minutes or longer	7 male and 7 female	20-46 yrs	14	Healthy	N/A	N/A

Observations:

No significant differences in mean values of any of the ventilatory parameters were found between the two free-breathing exposures. There were significant differences in the ventilatory pattern in the forced oral SO_2 exposures. Cross-over from nasal to oral/nasal ventilation occurred at a lower workload and minute ventilation following 30-minutes of oral only breathing ($p<0.001$). Cross-over occurred at an average workload of 435 kg.m/min following oral inhalation while cross-over occurred at 557, 557, and 607 kg.m/min for the free breathing in filtered air and SO_2 , and nasal breathing in SO_2 protocols, respectively.

The nasal component of the ventilation at cross-over following oral-only breathing was smaller ($p<.003$) then the other conditions, but the oral component was similar between encumbered and free-breathing conditions ($p>0.06$).

There was no differences in the pulmonary function parameters measured prior to exposure.

Following exposure, there was a significant ($p<0.0125$) pre-post decrease n peak flow independent of the exposure condition.

*Statistically significant

Author's Conclusions:

From our results we conclude that in normal ie nonasthmatic and non-hypersensitive or atopic subjects, exposure to 2 ppm SO_2 did not result in demonstrable changes in pulmonary parameters. It does appear, however, that forced oral breathing in 2 ppm SO_2 causes a change in the nasal airway dynamics so that the subjects start to breathe orally at a lower workload than in other exposure conditions. This would explain the significantly smaller ventilations prior to and at the cross-over. It is likely that the observed effects are due to forced oral breathing and not SO_2 because no other respiratory parameters were changed.

Review – Assessment:

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+/-)Are the subjects a representative sample of the population to which extrapolations will be made? (+/-) Was the method of recruitment appropriate? (No incentive info) Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-)Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? (+)Were the chosen exposure levels appropriate to investigate the primary objective? (+)Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-)Were both nominal and actual exposure concentrations recorded? (+)Was the duration of exposure appropriate to investigate the primary objective? (+)Was the duration of exposure precisely defined? (+/-)Was the mode of administration appropriate? (+)Was the frequency of administration appropriate to investigate the primary objective? (-)Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+)Was a full description of the monitoring devices provided? (+/-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+/-)Were the subjects randomly assigned to exposure groups? (N/A)Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+)Is the control regime appropriate? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+/-)Was the length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined?

	(+)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+/-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+/-)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+) Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables? (+)Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

No significant differences in mean values of any of the ventilatory parameters were found between the two free breathing exposures. There was no consistent effect of the SO_2 exposure on the subjects' pulmonary functions, including airway resistance. There were no pulmonary effects following 40 minutes or longer exposure to 2ppm SO_2 with continuous exercise in these subjects whether inhalation was via free breathing or forced oral or nasal breathing. This result was not consistent with results from other studies.

Forced oral breathing in 2 ppm SO_2 caused a change in the nasal airway dynamics so that the subjects start to breath orally at a lower workload than in other exposure conditions. The authors admit that the design used to minimize the number of repeat tests per subject does not allow the determination that the effects observed are related to SO_2 or to the forced oral breathing.

The results may be important for the design of exposure protocols investigating response to inhaled substances during exercise. However, care must be taken to insure that the mode of breathing (oral;nasal;oral/nasal) is the same for all subjects. Therefore, the results would be more comparable. In this study, it was discovered that three of the subjects were mouth breathers, while the preliminary tests determined that they were nose breathers. The methods used for the preliminary testing involved using an oral catheter that forced the three subjects to breath through their nose while they were actually mouth breathers. The crossover points were also variable, indicating that the assigned workload was not appropriate and individuals will always vary in their response.

Confidence index:

This study successfully limited confounding factors prior to the study by recording subject statistics such as age and gender. Furthermore, the study mentioned that subjects were made aware of possible experimental risks. However, no mention is given for why only 14 participants were used for the study, since more would have allowed results to be statistically significant. Furthermore, there is no information on random exposure. In addition, the cross-over point in subjects was variable. Some subjects hesitated or back and forth breathing occurred.

Review – Confidence index ranking:

Moderate

Author: Woodford DM, Coutu RE, Gaensler EA	**Internal ID: 269**
Title: Obstructive lung disease from acute sulfur dioxide exposure.	
Year: 1979	
Abstract: A previously healthy, non-smoking young man is presented who was briefly exposed to a high concentration of SO_2. An immediate episode of pulmonary edema was followed by a silent interval with subsequent development of a severe, irreversible obstructive syndrome. This history, together with radiographs showing hyperinflation and sequential physiologic studies indicative of obstruction without bronchospasm and without loss of parenchyma suggest bronchiolitis obliterans as the causative lesion. The literature concerning this syndrome and its relationship to toxic inhalation is briefly reviewed.	
Objective: To present a case report of a young man accidentally exposed to SO_2.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
n/a	**15-20min**	**Male**	**25yrs**	**1**	**healthy**	**n/a**	**n/a**

Observations:

Man noticed symptoms of burning and tearing eyes, rhinorrhea, cough, and 'almost passed out'. Physical examination revealed conjunctivitis, and fine, crepitant basilar rales. He was treated with oxygen and released. His chest roentgenogram was clear.
After a few days, he noted dyspnea with exertion and severe cough, which progressed until hospitalization was required.
Hyperinflation was found to be present, and the patient had coarse basilar rales, purulent sputum, and was dyspneic at rest.
Over the next 20months, the man was found to have persistent obstructive airways disease.
Three years post-exposure, symptoms were found to have stabilized but included forced expiratory wheezing, unchanged pulmonary function studies and chest film.

*Statistically significant

Author's Conclusions:

"Even though there was no histologic confirmation in our patient, we believe that bronchiolitis obliterans was the principle cause of his irreversible obstruction. The exposure, clinical course, radiologic changes and physiopathology were all compatible with this diagnosis."

Review – Assessment:

A. Subjects:	-✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ±✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Were identical 'criteria for exclusion' applied to both cases and controls?

	±✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	-✎ Was the exposure concentration measured? +✎ Is the exposure duration known?
C. Equipment:	±✎ Was a full description of the monitoring devices provided? ±✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+✎ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? ±✎ Were appropriate controls recruited? ±✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the timing and length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	±✎ Was the assessment identical for cases and controls? +✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✎ Was individual data reported for each subject? +/-✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	±✎ Are the statistical methods used appropriate? +/-✎ Are confidence intervals reported? ±✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? ±✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +✎ Were assumptions reasonable? ±✎ Are the methods reproducible? ±✎ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)?

	+✏ Do the conclusions make biological sense? +✏ Are the extrapolations reasonable? +✏ Is the paper in a peer-reviewed journal? +✏ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings document the symptoms and clinical progression of a man exposed to suspected high levels of SO_2, albeit for only a short period of time (15-20min). As a result of his exposure, he developed symptoms consistent with bronchiolitis obliterans, a condition from which he has never recovered. This case report is useful for documenting the effects of high-level exposures on healthy persons, and reminds one of the danger of high-level SO_2 exposure.

Confidence index:

Since this is a case report, it cannot be judged by the same criteria as a case-control study. In terms of a case-report study, assessment seems to be thorough, and follow-up was for a sufficient amount of time (3 years). The data presented here are very useful for examining human respiratory effects of high dose SO_2 exposure. Pulmonary function was assessed by multiple parameters. Recorded that the patient was previously healthy and a nonsmoker – limit of confounding factors. Symptoms are in agreement with other published works for persons acutely exposed. Did not record the actual exposure dose. Did not mention how the parameters for the serial lung function tests were measured.

Review – Confidence index ranking:

Low

Author: Charan, NB., Myers, CG., Lakshminarayan, S., Spencer, TM.

Internal ID: 270

Title: Pulmonary Injuries Associated with Acute Sulfur Dioxide Inhalation

Year: 1979

Abstract: The report describes an industrial accident in which 5 previously healthy persons were acutely exposed to very high concentrations of sulfur dioxide. Of five subjects, two with the highest exposure died immediately; histological examination of the lungs revealed sloughing of mucosal lining along with hemorrhagic alveolar edema. Three survivors were evaluated with pulmonary function tests at regular intervals. One subject eventually developed symptomatic severe airway obstruction that was unresponsive to bronchodilators, which another developed asymptomatic mild obstructive and restrictive disease. The third demonstrated normal pulmonary functions. This study documented histologic features of fatal exposure to sulfur dioxide and emphasized the need to consistently evaluate pulmonary function in non-fatal cases.

Objective: The objective of the study was to document the histologic features of fatal exposure to sulfur dioxide and to stress the need to follow parameters of pulmonary function in nonfatal cases.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
unknown	days 1, 50, 69, 116 after the explosion	male	25, 46, 51	3	Healthy, one smoker	-	-

Observations:

This study was anecdotal or descriptive in nature and no statistically significant results were reported. The following are general observations and conclusions:

The 2 persons with the highest exposure to SO_2 fumes died shortly after exposure. At postmortem examination, the predominant abnormalities were confined to the upper and lower respiratory tracts. The acute symptoms in the 3 survivors were identical and included irritation and soreness of the eyes, nose, and throat, tightness in the chest, and intense dyspnea. Eyes revealed severe conjunctivitis and superficial corneal burns. Pharyngeal mucosa was hyperaemic, but no ulcerations were seen. Examination of the chest revealed markedly decreased breath sounds, diffuse rales. Chest roentgenogram was normal with no evidence of edema. In subjects 4 and 5, arterial blood gas analysis revealed mild hyposemia and hypocapnia. Subject 3 was more hypoxemic, with an arterial PO_2 or 72 mm Hg when fraction of inspired O_2 was 0.6.

Results from serial pulmonary tests revealed Subject 3 developing severe irreversible airway obstruction with air trapping and hyperinflation. Subject 4 developed mild airway obstruction unresponsive to inhaled bronchodilators by day 116. Lung volumes revealed small decreases in functional residual capacity and residual volume when compared to initial values. Subject 5 had no pulmonary function abnormalities acutely or when tested serially.

*Statistically significant

Author's Conclusions:

This report serves to document the histologic features of fatal exposure to sulfur dioxide and stresses the need to follow parameters of pulmonary function in the nonfatal cases. It serves to emphasize the damaging effects of SO_2 inhalation and the possibility of developing irreversible airway obstruction after nonfatal exposures to SO_2.

The deterioration in spirometric data noted after the accident suggests that the obstruction was a sequel to the exposure. Absence of airway obstruction in Subject 5 might have been due to his minimal exposure. This report serves to emphasize the damaging effects of SO_2 inhalation and the possibility of developing irreversible airway obstruction after nonfatal accidental exposures to SO_2 and other water-soluble noxious gases.

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? N/A Were identical 'criteria for exclusion' applied to both cases and controls? N/A Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	N/A Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(+) Was a full description of the monitoring devices provided? (+) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? N/A Were appropriate controls recruited? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	N/A Was the assessment identical for cases and controls? N/A Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams?

	(+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? N/A Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+/-) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+/-) Did the investigator appropriately interpret the statistics? (+/-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+/-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (+) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study assessed the victims of a mine explosion for pathological effects of SO_2 exposure. Consequently, it was largely a descriptive study, with no statistical analysis. Nevertheless, physiological responses of subjects further highlight the damaging effects of SO_2 inhalation and the possibility of developing irreversible airway obstruction after non-fatal accidental exposures to SO_2 and other water-soluble noxious gases.

Two of the subjects died immediately following the accidental exposure. The remaining 3 subjects were examined 1, 50, 69 and 116 days after the exposure. The acute symptoms in the 3 survivors were identical and included irritation and soreness of the eyes, nose, and throat, tightness in the chest, and intense dyspnea Examination of the chest revealed markedly decreased breath sounds, diffuse rales and rhonchi. Subject 4 (smoker, 46 yr. old) did not have airway obstruction on the first day but developed mild airway obstruction unresponsive to inhaled bronchodilators by day 116. His lung volumes revealed small decreases in functional residual capacity and residual volume when compared to his initial values. Subject 5 (25 yr old) had no pulmonary function abnormalities either acutely or when tested serially. Subject 3 (51 yr old) continues to have dyspnea on mild exertion. The 3 survivors completely recovered from the eye injuries within a few weeks.

Confidence index:

This study provides a good description of the pulmonary pathology caused by massive exposure to SO_2. Within the materials and methodology section, the author mentions confounding factors such as smoking that may have had an earlier effect on lung function. Since only 3 subjects were assessed in the study, results cannot be extrapolated with scientific certainty. Despite this, this report serves to highlight the serious danger associated with SO_2, an important study in the likely event of more accidental exposures. However, statistically significant results are not possible due to the sample size. The above findings indicate that the three survivors all had the same acute symptoms but different longer term symptoms. The subjects differed in age, smoking history, and exposure level. For example, subject 4 was wearing a dual-cartridge chemical mask and hence had less exposure than the other subjects. Subject 5 had no pulmonary function abnormalities either acutely or when tested serially. However, he was also the youngest subject and a firemen, so presumably was in better physical health compared to the other subjects. Subject 3 continued to have health problems by day 116, but was also the oldest subject. This study is primarily useful for the formulation of hypotheses that can be tested subsequently using an analytic design. However, a major downfall of the study is the lack of information on exposure concentration.

Review – Confidence index ranking:

Low

DRAFT

Author: Galea M	**Internal ID: 271**
Title: Fatal sulfur dioxide inhalation	
Year: 1964	
Abstract: The histological findings in a 35-year old man dying 17days after accidental inhalation of SO_2 fumes are presented. Some of the similarities, clinical and microscopic, observed in cases of sulfur dioxide inhalation are discussed.	
Objective: "The following report concerns an unfortunate accident arising from accidental inhalation of sulfur dioxide fumes by two men for an interval of about 15 to 20 min in a local pulp and paper plant."	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
n/a	**15-20min**	**Male**	**a) 45 b) 35**	**2**	**a) heavy smoker b) healthy**	**n/a**	**n/a**

Observations:

Four months following exposure, the 45 year old heavy smoker showed evidence of hyperventilation at rest and an elevated ventilatory equivalent, indicating marked respiratory fatigue. His vital capacity was satisfactory, but a delayed chronometric vital capacity and prolonged expiratory phase were observed.

The 35 year old patient was discharged several days following exposure with a sufficiently satisfactory clinical condition. Ten days thereafter he was readmitted complaining of dry, irritable and tiresome cough, dyspnea and copious amount of mucus. He had rales on both lung bases and wheezing was audible. He died 17 days after exposure. Upon autopsy, both lungs were uniformly of striking marble appearance, voluminous, feathery and pillowy in consistency. No elastic rebound was elicited on compression but no appreciable edema either externally or on serial sectioning. Some thick mucus was present just proximal to the tracheal bifurcation. The gross impression was that of a diffuse type of acute emphysema. The histological findings were characterized by 1) an extensive tracheobronchitis, 2) intense activity of the mucus secreting acini of the submucosal glands of the trachea, 3) airway lesions and 4) distension of alveoli and air sacs with rupture of many alveolar septa.

*Statistically significant

Author's Conclusions:

"The fundamental lesion of extensive peribronchiolar fibrosis and bronchiolitis obliterans was presumably responsible for the acute emphysematous changes consistent with the immediate cause of death."

Review – Assessment:

A. Subjects:	-✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate

	for the objective of the study? ±✎ Are the subjects a representative sample of the population to which extrapolations will be made? ±✎ Were identical 'criteria for exclusion' applied to both cases and controls? ±✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	-✎ Was the exposure concentration measured? +✎ Is the exposure duration known?
C. Equipment:	-✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+/-✎ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? +/-✎ Were appropriate controls recruited? -✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the timing and length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	±✎ Was the assessment identical for cases and controls? +✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +/-✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✎ Was individual data reported for each subject? ±✎ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	±✎ Are the statistical methods used appropriate? ±✎ Are confidence intervals reported? ±✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
G. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +/-✎ Were assumptions reasonable? +✎ Are the methods reproducible?

	±✎ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? +✎ Do the conclusions make biological sense? +✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The paper describes the clinical course and autopsy results of a man exposed to high levels of SO_2. The results are useful for understanding the pathology of SO_2 exposure and developing methods of treating it. Acute exposure of two workers to SO_2 resulted in very different outcomes. For the 45 year old patient it was inferred that he would manifest no dyspnea at rest, but his capacity for exertion would be markedly restricted. For the 35 year old patient the fundamental lesion of extensive peribronchiolar fibrosis and bronchiolitis obliterans was presumably responsible for the acute emphysematous changes consistent with immediate cause of death. The health of the patients prior to exposure was not a factor in their response.

Confidence index:

While this paper was anecdotal, subjects were adequately characterized. Although materials and methods are not included for this kind of study, observations were adequately characterized. Limited exam prior to death – did not discuss pulmonary function tests as for the 45 year old patient, but an extensive histological exam was performed at autopsy. Changes were only observed in respiratory organs as expected. No suggestions of potential therapeutic approach to take given the nature of the symptoms. No literature cited of previous acute SO_2 exposures, but did discuss toxicity of other chemicals including cadmium oxide and sulfur dioxide.

Review – Confidence index ranking:

Low

Author: Rabinovitch, S., Greyson, ND., Weiser, W. and Hoffstein, V.	**Internal ID:** 272

Title: Clinical and Laboratory Features of Acute Sulfur Dioxide Inhalation Poisoning; Two-year Follow-Up

Year: 1989

Abstract: We present clinical and laboratory results (including nuclear imaging) obtained over a period of two years in two nonsmoking miners who were exposed to high concentrations of sulfur dioxide after a mine explosion. Within 3 wk of the accident, both miners had evidence of severe airways obstruction; hypoxemia, markedly reduced exercise tolerance, ventilation-perfusion mismatch, and evidence of active inflammation as documented by positive gallium lung scan. Serial ventilation-perfusion scans over the first 12 months showed progressive improvement without returning to normal. After the initial recovery, there has been no significant change over the subsequent two years post injury. Pulmonary function and exercise tests also displayed a similar pattern of initial improvement. We conclude that 1. acute exposure to high concentrations of SO_2 results in severe airways obstruction, 2. pulmonary function abnormalities are partially reversible, and 3. most of the improvement occurs within 12 months after the initial injury.

Objective: To examine clinical and laboratory features of acute sulfur dioxide inhalation poisoning.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
>40 ppm	3.5 hours	male	25, 36 years	2	healthy	unknown	unknown

Observations:

Within 3 wk of the accident, both miners had evidence of severe airways obstruction, hypoxemia, markedly reduced exercise tolerance, ventilation-perfusion mismatch, and evidence of active inflammation as documented by positive gallium lung scan. Serial ventilation-perfusion scans over the first 12 months showed progressive improvement without returning to normal. After the initial recovery, there has been no significant change over the subsequent two years post injury. Pulmonary function and exercise tests also displayed a similar pattern of initial improvement.

*Statistically significant

Author's Conclusions:

"We conclude that 1. Acute exposure to high concentrations of SO_2 results in severe airways obstruction, 2. Pulmonary function abnormalities are partially reversible, and 3. Most of the improvement occurs within 12 months after the initial injury. The severe hypoxemia observed was secondary to ventilation perfusion mismatch and exertional dyspnea was due to ventilatory limitation."

Review – Assessment:

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study?

	(+/-) Are the subjects a representative sample of the population to which extrapolations will be made? N/A Were identical 'criteria for exclusion' applied to both cases and controls? N/A Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(-) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(+/-) Was a full description of the monitoring devices provided? (+/-) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+/-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? N/A Were appropriate controls recruited? N/A Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	N/A Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? N/A Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	N/A Are the statistical methods used appropriate? N/A Are confidence intervals reported? N/A Did the investigator appropriately interpret the statistics? N/A Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+/-) Are the results novel? N/A Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? N/A Are the methods reproducible? (+/-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)?

	. (+) Do the conclusions make biological sense? . (+) Are the extrapolations reasonable? . (+) Is the paper in a peer-reviewed journal? . (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results indicate that severe airway obstruction in subjects slowly improved but did not completely resolve over a two year period. The author recommends long-term follow-up to predict the extent of recovery of pulmonary function.

Confidence index:

This study exhibited fairly extensive testing of pulmonary function, exercise performance and arterial blood gases – all data was given for a 2 yr period of assessment. Both miners were observed to be healthy and nonsmokers prior to exposure, limiting confounding factors. Furthermore, the data provided were in agreement with what was observed following acute exposures. While this paper was anecdotal in nature, the subjects involved, as well as the monitoring techniques used were explicitly described. However, no confidence intervals were reported. In addition, the number of subjects was small and the exposure concentrations were not well-characterized.

Review – Confidence index ranking:

Moderate to low

Author: Leung KH. Post GB. Menzel DB.	**Internal ID: 273**
Title: Glutathione S-sulfonate, a sulfur dioxide metabolite, as a competitive inhibitor of glutathione S-transferase, and its reduction by glutathione reductase.	
Year: 1985	
Abstract: Glutathione S-sulfonate ($GSSO_3H$) is a reaction product of glutathione disulfide (GSSG) and sulfite, the hydrated form of sulfur dioxide. In the present study, GSSO3H was found to be a potent competitive inhibitor of the glutathione S-transferases (GST) in the rat liver (Ki = 14 microM) and lung (Ki = 9 microM), and in human lung tumor-derived A549 cells (Ki = 4 microM). $GSSO_3H$ was also reduced by a cytosolic enzyme in the rat liver (Km = 313 microM) and lung (Km = 200 microM), and human lung A549 cells (Km = 400 microM). These results suggest that SO_2 may affect the detoxification of xenobiotic compounds by inhibiting, via formation of $GSSO_3H$, the enzymatic conjugation of glutathione (GSH) and reactive electrophiles. Although $GSSO_3H$ can be enzymatically degraded, the high substrate Km value suggests that this compound may not be readily reduced at low concentrations.	
Objective: "In the present study, we have found that $GSSO_3H$ is a strong competitive inhibitor of glutathione S-transferase activity in the rat liver and lung, and in human lung tumor-derived A549 cells. We have also shown that $GSSO_3H$ can be enzymatically degraded by a cytosolic reductase present in these tissues."	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
n/a	**Single dose**	**Rat lungs / human lung tumor cells**	**Sprague-dawley**	**n/a** **200-250g**	**Male**	**n/a**	**n/a**	**n/a**	**n/a**

Observations:

In the present study, $GSSO_3H$ was found to be a potent competitive inhibitor of the glutathione S-transferases in the rat liver and lung, and in human lung tumor-derived A549 cells. $GSSO_3H$ was also reduced by a cytosolic enzyme in the rat liver and lung, and human lung. These results suggest that SO_2 may affect the detoxification of xenobiotic compounds by inhibiting, via formation of $GSSO_3H$, the enzymatic conjugation of glutathione (GSH) and reactive electrophiles. Although $GSSO_3H$ can be enzymatically degraded, the high substrate K_m value suggests that this compound may not be readily reduced at low concentrations.
*Statistically significant

Author's Conclusions:

"In the present study, we have shown that $GSSO_3H$, the reaction product of hydrated SO_2 and GSSG, is a strong competitive inhibitor of both cytosolic and microsome-associated GST in the rat liver and lung and in human lung-derived A549 cells. This result suggests that SO_2 may affect the detoxification of PAH by inhibiting the enzymatic conjugation of GSH and the reactive electrophiles via formation of $GSSO_3H$. The effects of SO_2 and $GSSO_3H$ on the metabolism of BP is currently under investigation."

Review – Assessment:

A. Test animals:	±✎ Is the number of animals sufficient to produce statistically significant results? ☐ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.*

	✏ Are animal characteristics adequately recorded and appropriate for the objective of the study? +/-a) Specie ☐ *Guideline: Rat, or justification for use of another mammalian specie.* ±b) Age ☐ *Guideline: Adult, i.e.,8-12 weeks.* +/-c) Sex ±d) Pre-test health ☐ *Guideline: Females should be nulliparous and non-pregnant.* +/-e) Weight variation ☐ *Guideline: Below 20% variation* ±f) Source ☐ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	+✏ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? ±✏ Was there a gradient of exposure levels? ☐ *Guideline: Three exposure levels in addition to control.* ±✏ Were the chosen exposure levels appropriate to investigate the primary objective? ±✏ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? ±✏ Were both nominal and actual exposure concentrations recorded? ±✏ Was the duration of exposure appropriate to investigate the primary objective? ±✏ Was the duration of exposure precisely defined? ±✏ If exposure chambers were employed, were animals exposed during the equilibration period? ☐ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* ±✏ Was the mode of administration appropriate? ±✏ Was the frequency of administration appropriate to investigate the primary objective? ±✏ Was evidence provided to justify the dosing regime? ±✏ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	±✏ Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ☐ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* ±✏ Was the number of animals grouped in a single chamber stated? ☐ *Guideline: Groupings should permit clear observation of each animal.* ±✏ Was the manner by which animals were grouped appropriate? ☐ *Guideline: Animals may be grouped by sex, or individually.* ±✏ Was the type and source of feed and water stated? Was it appropriate? ☐ *Guideline: Conventional lab diet* ±✏ Was the amount and feeding schedule specified?

	☐ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	±✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ☐ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* ±✎ Was a full description of the monitoring devices provided? ±✎ What was the sensitivity of the monitoring devices employed?
E. Procedural:	±✎ Was the acclimation period specified? Was it of acceptable duration? ☐ *Guideline: 5 days* ±✎ Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? ±✎ Were test animals randomly assigned to exposure groups? ±✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) ±✎ Was a control group assayed? ±✎ Is the control regime appropriate? ±✎ Was the period of observation following exposure appropriate? ☐ *Guideline: Longer than 14 days.*
F. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical? +✎ Was raw data provided to permit the reader to arrive at his/her own conclusions? -✎ Was individual data reported for each test animal?
G. Data analysis:	±✎ Are the statistical methods used appropriate? -✎ Are confidence intervals reported? ±✎ Did the investigator appropriately interpret the statistics? ±✎ Are the observed trends statistically significant?
H. Interpretations:	+✎ Was the original objective addressed? +✎ Are the results novel? +✎ Was the design of the study appropriate to investigate the primary objective? +/-✎ Were assumptions reasonable? +/-✎ Are the methods reproducible? +✎ Did either the study design or the statistical analysis limit confounding variables? +✎ Do the conclusions make biological sense?

	+✎ Are the extrapolations reasonable? +✎ Is the paper in a peer-reviewed journal? +✎ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study evaluated the effect of GSSO3H on the activity of GST isolated from pooled rat livers, pooled rat lungs and human A549 cells. The results clearly shows that GSSO3H is a potent competitive inhibitor of GST activities.The GSH pathway which is responsible for detoxification of benzo(a)pyrene may be vulnerable to interference by SO_2 since the latter can react with GSSG to form $GSSO_3$, resulting in depletion of the limited GSH supply in the lung.

Confidence index:

This is an in vitro study assessing the effects of a potential sulfur dioxide metabolite. There is no evidence that the actual inhibition of GST activities occurs in vivo following exposure to SO_2. There is no mention of the amount of $GSSO_3$ that is produced in the lungs or livers of exposed animals/humans and whether the concentrations used in vitro for the competitive assays are even relevant. Furthermore, it is unclear what the pre-trial conditions were like for rats, or what the type of food was. It is not clear if a control group was assayed, and if so, what the control regime was.

Review – Confidence index ranking:

Low

Author: Koenig JQ. Covert DS. Hanley QS. van Belle G. Pierson WE. **Internal ID: 277**

Title: Prior exposure to ozone potentiates subsequent response to sulfur dioxide in adolescent asthmatic subjects.

Year: 1990

Abstract: The objective of this study was to test whether prior exposure to a low concentration of ozone (120 ppb) would condition airways in asthmatic subjects to respond to a subthreshold concentration of sulfur dioxide (100 ppb). Eight male and five female subjects 12 to 18 yr of age participated. They all had allergic asthma and exercise-induced bronchospasm. Subjects were exposed to three test atmosphere sequences during intermittent moderate exercise (a 45-min exposure to one pollutant followed by a 15-min exposure to the second pollutant). The sequences were: air followed by 100 ppb SO_2, 120 ppb O_3 followed by 120 ppb O_3, and 120 ppb O_3 followed by 100 ppb SO_2. The pulmonary function measurements assessed were FEV1, total respiratory resistance (RT), and maximal flow (Vmax50). Air-SO_2 and O_3-O_3 exposures did not cause significant changes in pulmonary function. On the other hand, exposure to 100 ppb SO_2 after a 45-min exposure to 120 ppb O_3 caused a significant (8%) decrease in FEV1 ($p = 0.046$), a significant (19%) increase in RT ($p = 0.048$), and a significant (15%) decrease in Vmax50 ($p = 0.008$). It is concluded that prior O_3 exposure increased bronchial hyperresponsiveness in these subjects such that they responded to an ordinarily subthreshold concentration of SO_2. These data suggest that assessment of pulmonary changes to single pollutant challenges overlooks the interactive effects of common coexisting or sequentially occurring air pollutants.

Objective: "In this present study, we show that prior exposure to ozone (O_3) potentiates the pulmonary response to a subsequent exposure to a subthreshold dose of sulfur dioxide (SO_2).

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
100 ppb	**15min**	**8M 5F**	**12-18**	**13**	**Allergic asthmatic**	**n/a**	**100ppb**

Observations:

"Pulmonary function values 2 to 3min post-exercise (PE1) compared with baseline values showed that FEV1 decreased 3% after air-SO_2 exposure, 2% after O_3-O_3 exposure, and 8% after O_3-SO_2 exposure. RT increased 14% after air-SO_2 exposure, 0% after O_3-O_3 exposure, and 20% after O_3-SO_2 exposure. Vmax50 decreased 5% after air-SO_2 exposure, 8% after O_3-O_3 exposure, and 15% after O_3-SO_2 exposure… The analysis of variance test showed that pulmonary function changes after O_3-SO_2 exposure were significantly different from those after either air-SO_2 or O_3-O_3 exposure. The p-values were 0.046 for FEV1, 0.048 for RT, and 0.008 for Vmax50."

*Statistically significant

Author's Conclusions:

"In conclusion, our study has shown that sequential exposure to O_3 and SO_2 at realistic ambient concentrations affected pulmonary mechanics in adolescent subjects with asymptomatic asthma who were under appropriate clinical care. These data suggest that assessment of pulmonary changes to single pollutant challenges may not detect, and thus underemphasize the interactive effects of common air pollutants."

Review – Assessment:

A. Subjects:	+✎ Is the number of subjects sufficient to produce statistically significant results? +✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? +✎ Are the subjects a representative sample of the population to which extrapolations will be made? +✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? -✎ Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	+✎ Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? -✎ Was there a gradient of exposure levels? +✎ Were the chosen exposure levels appropriate to investigate the primary objective? -✎ Were previous preliminary trials conducted to identify the range of appropriate exposure levels? -✎ Were both nominal and actual exposure concentrations recorded? +✎ Was the duration of exposure appropriate to investigate the primary objective? +✎ Was the duration of exposure precisely defined? +✎ Was the mode of administration appropriate? +✎ Was the frequency of administration appropriate to investigate the primary objective? -✎ Was evidence provided to justify the dosing regime? +✎ Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	+✎ Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined +✎ Was a full description of the monitoring devices provided? -✎ What was the sensitivity of the monitoring devices employed?
D. Procedural:	+/-✎ Were the subjects randomly assigned to exposure groups? +/-✎ Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) -✎ Was a control group assayed? +✎ Is the control regime appropriate? +✎ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) +✎ Was the length of follow-up appropriate? +✎ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	+✎ Were appropriate endpoints examined? +✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) +✎ Were accepted assessment methods employed? +✎ Were assessment measures sufficiently sensitive? +✎ Was the nature and severity of symptoms graded against an explicitly defined scale? +✎ Did qualified personnel conduct the exams? +✎ Was the timing and frequency of assessment logical?

	+✓ Was raw data provided to permit the reader to arrive at his/her own conclusions? +✓ Was individual data reported for each subject? ±✓ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	+✓ Are the statistical methods used appropriate? ±✓ Are confidence intervals reported? +✓ Did the investigator appropriately interpret the statistics? +✓ Are the observed trends statistically significant?
G. Interpretations:	+✓ Was the original objective addressed? +✓ Are the results novel? +✓ Was the design of the study appropriate to investigate the primary objective? +✓ Were assumptions reasonable? +✓ Are the methods reproducible? +✓ Did either the study design or the statistical analysis limit confounding variables? +✓ Do the conclusions make biological sense? +✓ Are the extrapolations reasonable? +✓ Is the paper in a peer-reviewed journal? +✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study demonstrates that sequential exposure to ozone and then SO_2 at realistic ambient concentrations affected pulmonary mechanics in adolescent subjects with asymptomatic asthma who were under appropriate care; sensitization of airways to a subthreshold concentration of SO_2 in adolescent subjects with asthma. This is in contrast with previous studies that showed that similar adolescent subjects with asthma showed no significant changes. The author concludes that assessment of pulmonary changes to single pollutant challenges may not detect, and thus underemphasize the interactive effects of the common air pollutants.

Confidence index:

The extrapolation is unwarranted since did not look at the effect of ozone plus SO_2 or SO_2 followed by SO_2 sequences. Also exposures were performed with mouthpieces prevented nasal scrubbing, trying to limit confounding variables but also don't reflect accurate real exposure conditions. Exposure conditions and assessment methods were well defined – study should be easily reproducible. Experiment was also blinded by having the person calculating pulmonary function tests did not know the test atmosphere. Sufficient sample size to identify statistically significant changes. Also had subjects complete a symptoms rating test after each exposure, sequence of exposures was random and tests were given at least a week apart. Subjects were each positive for a methacholine challenge. Most subjects were on medication and were allowed to take medication up to 4 hours before an exposure.

Review – Confidence index ranking:

Moderate to high

NON-CLINICAL REVIEW FORM

Author: Hilado, C.J., Machado, A.M.	**Internal ID:** 284
Title: Effect of Sulfur Dioxide on Swiss Albino Mice	
Year: 1977	
Abstract: Times to incapacitation and death and LC_{50} values were determined for male Swiss albino mice exposed to different concentrations of sulfur dioxide in a 4.2 liter hemispherical chamber. The LC_{50} for a 30 minute exposure was about 3000 ppm SO_2.	
Objective: This paper presents sulfur dioxide data determined for male Swiss albino mice.	

Overall study design.

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
1100-14300ppm	Up to 30min; termination upon death of all animals	Mice	Swiss Albino	No info	Male	Not clear	No info	1190	

Observations:

Time to first incapacitation was less than 3 min over the range of SO_2 concentrations from 3500-14300ppm and increased to about 6 minutes as SO_2 was reduced toward 1100ppm.

As SO2 concentration was reduced from 14,300 ppm to 3,500, the average time to staggering increased from 1 minute to 6 minutes. With this decrease in SO2 concentration, the average time to convulsions increased from 2 minutes to 8 minutes.

Average time to death increased from approximately 3 min to 8 min as SO_2 concentrations decreased from 14, 300 to 4800 ppm. At 3570ppm SO_2, only 50% mortality was obtained over the 30 min exposure, but most surviving animals died within 3 days of the exposure period. This concentration would approximate the 30 min LC_{50} if defined so as to be limited to deaths with the 30 min exposure. At 2380ppm SO_2, mortality averaged 38 percent during the 30min exposure, with no delayed deaths. There were no deaths during or after 30 min exposure to 1190 ppm SO_2.

The 5min, 10min and 15min LC_{50} values for $SO_2$are 6800ppm, 4400ppm, and 4000ppm respectively and the 30 min LC_{50} is 3000ppm.

*Statistically significant

Author's Conclusions:

Sulfur dioxide appears to be more toxic to Swiss albino mice than carbon monoxide in short exposures (5 min) and not significantly different from carbon monoxide in longer exposures (10-30min).

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	**(-) not clear how many** Is the number of animals sufficient to produce statistically significant results? ↳*Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie ↳ *Guideline: Rat, or justification for use of another mammalian specie.* **(no info)** b) Age ↳ *Guideline: Adult, i.e.,8-12 weeks.* **(-) male only** c) Sex d) Pre-test health **(no health info)** ↳ *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation ↳ *Guideline: Below 20% variation* f) Source **(-) no info** ↳*Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) **no control**Was there a gradient of exposure levels? ↳ *Guideline: Three exposure levels in addition to control.* (-) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (-) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (-) Was the duration of exposure precisely defined? **(no info)** If exposure chambers were employed, were animals exposed during the equilibration period? ↳ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate?

	(+) Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(no info)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? **(-) no info** *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* Was the number of animals grouped in a single chamber stated? **(-) no info** *Guideline: Groupings should permit clear observation of each animal.* Was the manner by which animals were grouped appropriate? **(-) no info** *Guideline: Animals may be grouped by sex, or individually.* Was the type and source of feed and water stated? Was it appropriate? **(-) no info** *Guideline: Conventional lab diet* Was the amount and feeding schedule specified? **(-) no info** *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. **(some detail)** *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(-)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	Was the acclimation period specified? Was it of acceptable duration? **(no info)** *Guideline: 5 days* **(-)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(-)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to

	the mechanism used to generate random assignment) **(-)** Was a control group assayed? **(n/a)** Is the control regime appropriate? Was the period of observation following exposure appropriate? **(+)** *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? **(some)** Was individual data reported for each test animal?
G. Data analysis:	(-) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (-) Did the investigator appropriately interpret the statistics? **(no info)** Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? **(no extrapolations made)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

It was determined that the LC_{50} for a 30 minute exposure was about 3000ppm SO_2. There was no further analysis on the animals after death.

This study is novel in that it presents the LC50 values for exposure of Swiss albino mice to sulfur dioxide. However, the author admits that the data may not be applicable to humans. Consequently, the authors original query regarding the time available for humans to escape from fire environments, may not be answered using this study. Furthermore, because the sample size is not given, it is unclear whether results may accurately represent a population or not.

The results from this test show a negative exponential relationship between SO_2 concentration and average time to death. A comparison is also done between the effects of SO_2 and carbon monoxide. The study suggests that sulfur dioxide appears to be significantly more toxic than carbon monoxide for exposure periods of up to five minutes at which point CO becomes more toxic. This may be because SO_2 contributes to respiratory tract irritation whereas CO targets the central nervous system.

Confidence index:

This study contained very limited information.

There was no information on the test animals or housing and feeding and only very limited information on exposure.

The mice were all male.

It is not clear how many mice were tested (sample size). It might be four, but it is not clear.

There is no information on data analysis.

The concentrations of $SO_2$are very high.

The exact gradient of exposures is not clear.

The initial health of the animals is not given, nor is the acclimation period, if any.

While some confounding factors are accounted for, such as weight and gender, the age of the mice is not stated. However, the author details the procedure, and precisely defines the periods of exposure and endpoints (eg. Staggering, convulsions and death).

A proper control was not established

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM

Author: Frank, N.R., Yoder, R.E., Yokoyama, E., Speizer, F.E.	**Internal ID:** 286

Title:The Diffusion of $^{35}SO_2$ From Tissue Fluids into the Lungs Following Exposure of Dogs to $^{35}SO_2$

Year:1967

Abstract: The surgically isolated airways of the head and upper neck of anesthetized, paralyzed dogs were exposed to 22±2 ppm of $^{35}SO_2$, delivered at a rate of 3.5 L/min for 30-60 min. The lungs were ventilated with ambient air by a positive displacement respiratory pump. In ten out of twelve measurements, over 95 per cent of the $^{35}SO_2$ administered to the isolated upper airways was found to be absorbed by the mucosa. In five out five animals, $^{35}SO_2$ was present in one or more expired gas samples collected either at the carina or from a bronchus; this expired $^{35}SO_2$ could not have entered the lower airways in inspired air and its presence provided evidence that the lungs were releasing the gas in expiration, presumably from the pulmonary capillaries. ^{35}S was present in the blood and urine within minutes of the onset of exposure to $^{35}SO_2$.

Objective: To examine the diffusion of $^{35}SO_2$ From Tissue Fluids into the Lungs Following Exposure of Dogs to $^{35}SO_2$. The uptake also varied during the throughout the exposure some of the dogs.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
22 ±2 ppm	30-60 min	Dogs	mongrel	No info	No info	6	No info	n/a	n/a

Observations:

Uptake of $^{35}SO_2$ by the isolated upper airways (n=5)

The uptake varied between the dogs. The absorptive rates varied between the dogs. After 25-30 min, 83-100% of the $^{35}SO_2$ was taken up by the isolated airways.

^{35}S in expired gas (n=5)

During the exposure of the isolated upper airways to $^{35}SO_2$, ^{35}S appeared in expired gas at the bronchial and carinal levels in at least one sampling **period** for each animal. The concentrations were variable and low representing less than 1 per cent of the exposure level. There were no consistent differences at the two sites.

^{35}S in blood (n=5)

^{35}S appeared in blood within 5 min of the onset of exposure. The level of radioactivity in blood increased throughout exposure becoming maximal at or near the end of exposure.

^{35}S in urine (n=3)

Generally, during exposure, the concentration of urinary **^{35}S** was 10 to 20 times greater than that of blood.

In ten out of twelve measurements, over 95 per cent of the SO_2 administered to the isolated upper airways was found to be absorbed by the mucosa. In five out of five animals, SO_2 was present in one or more expired gas samples collected either at the carina or from a bronchus; this expired SO_2 could not have entered the lower airways in inspired air and its presence provided evidence that the lungs were releasing the gas in expiration, presumably from the pulmonary capillaries. S

was present in the blood and urine within minutes of the onset of exposure to SO_2. The level of radioactivity in blood increased throughout exposure becoming maximal at or near the end of exposure and it fell slowly during the two-hour period following.
*Statistically significant

Author's Conclusions:

"We consider that the presence of ^{35}S in most of the bronchial and carinal samples is evidence that a gaseous form of ^{35}S leaves the tissue fluids of the lungs and enters the airways…^{35}S becomes significantly more concentrated in lung tissue when administered as a gas to either the intact upper airways or to an isolated segment of trachea than if it is injected parenterally as a salt."

The near-total uptake of SO2 by the isolated upper airways is in accord with earlier observations made in human subjects and experimental animals.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	Is the number of animals sufficient to produce statistically significant results? (-) *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? a) Specie (-) *Guideline: Rat, or justification for use of another mammalian specie.* b) Age (no info) *Guideline: Adult, i.e.,8-12 weeks.* (no info) c) Sex (no info) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) greater than 20%e) Weight variation *Guideline: Below 20% variation* (-) no info) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? Was there a gradient of exposure levels? (-) *Guideline: Three exposure levels in addition to control.* (more info needed) Were the chosen exposure levels appropriate to investigate the primary objective?

	(-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(-)** Were both nominal and actual exposure concentrations recorded? **(+)** Was the duration of exposure appropriate to investigate the primary objective? **(-)** Was the duration of exposure precisely defined? **(n/a)** If exposure chambers were employed, were animals exposed during the equilibration period? └ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(-)** Was the mode of administration appropriate? **(+)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(+)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(no info)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? └ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(n/a)** Was the number of animals grouped in a single chamber stated? └ *Guideline: Groupings should permit clear observation of each animal.* **(n/a)** Was the manner by which animals were grouped appropriate? └ *Guideline: Animals may be grouped by sex, or individually.* **(no)** Was the type and source of feed and water stated? Was it appropriate? └ *Guideline: Conventional lab diet* **(no info)** Was the amount and feeding schedule specified? └ *Guideline: During housing, ad libitum with unlimited water: During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(+)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. └ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure*

	(< 5mm of water) **(+)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(no info)** Was the acclimation period specified? Was it of acceptable duration? ↳ *Guideline: 5 days* **(no info)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(no info)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(-)** Was a control group assayed? **(n/a)** Is the control regime appropriate? **(-)** Was the period of observation following exposure appropriate? ↳ *Guideline: Longer than 14 days.*
F. Data collection:	**(+)** Were appropriate endpoints examined? **(-)** Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a no symptoms)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? **(-)** Was the timing and frequency of assessment logical? **some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(some)** Was individual data reported for each test animal?
G. Data analysis:	**(-)**Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(more info needed)** Did the investigator appropriately interpret the statistics? **(no info)** Are the observed trends statistically significant?
H. Interpretations:	**(+)** Was the original objective addressed? **(-)** Are the results novel?

	(-) Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (n/a) Did either the study design or the statistical analysis limit confounding variables? **(more detail needed)** Do the conclusions make biological sense? **(no extrapolations)** Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (no() Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Clinical or toxicological significance cannot be determined from the findings in this study due to weaknesses in the study design.

This study was novel in that it measured the uptake of ^{35}S by blood, its distribution within the blood and its elimination by the kidneys. This study was further novel in that it corrected for the absorption coefficient of the blood, something that previous studies had not done.

Results from this study indicate that the release of ^{35}S from tissue fluids represent an additional mode of exposure for the lungs. It suggests that the accumulation of ^{35}S in lung tissue following inhalation is due, in part, to its diffusion out of blood where the concentration of ^{35}S is 5 to 18 percent.

Confidence index:

There was no information on the test animals (age and sex) or housing and feeding. There was practically no information on exposure conditions.

There was very limited detail on the procedure,.

The number of animals used was not sufficient to produce results that can be extrapolated to a larger population. There were only six dogs and not all of them were used for each experiment.

For the urine analysis, only the results of one dog were shown.

The weight variation was greater than 20%.

There was no statistical analysis.

The introduction and literature review was inadequate.

For five of the dogs, the exposure lasted 30-38 min and for one dog it lasted 60 min.

Inhalation was not natural. The dogs were anaesthetized, and the airways of the nose, pharynx, larynx and uppermost trachea were surgically isolated from the respiratory symptoms. The mouth was closed with tape.

No mention was made of whether the dogs were acclimatized or what pre-test conditions were, and a control group was not assayed.

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM

Author: Min, Y.G., Rhee, C.S., Choo, M.J., Song, H.K., Hong, S.C.	**Internal ID:** 287

Title:Histopathologic Changes in the Olfactory Epithelium in Mice After Exposure to Sulfur Dioxide

Year:1994

Abstract: To investigate the effects of sulfur dioxide (SO_2) on olfactory epithelium, an experiment was performed with 56 mice from the same colony. Experimental animals were divided into three groups consisting of a 30-min exposure group (group 1), a 60-min exposure group (group 2), and a 120-min exposure group (group 3). The olfactory mucosa in these mice were studied by light microscopy immediately, and after 24 h, 48 h, or 72 h exposure to 20 ppm of SO_2. Edema, loss of cilia, epithelial thinning, and epithelial desquamation in the olfactory epithelium were observed in groups 2 and 3. The basal lamina and the connective tissue were well preserved throughout the entire mucosa. Injuries to olfactory epithelium became severer with exposure time. These changes were further pronounced 24 h after exposure. Regenerated epithelia were not observed in any group. Scanning electron microscopic findings were consistent with light microscopic findings. Olfactory epithelial surface were consistent with light microscopic findings. Olfactory epithelial surface was sloughed off and revealed, underlining intact basal lamina. The results of this study suggest that early lesions of olfactory epithelium after exposure to SO_2 may be primarily degenerative.

Objective: This study was conducted to analyze possible histopathologic changes in the mouse olfactory epithelium after exposure to 20 ppm SO_2 and the recovery pattern.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
20 ppm	Three groups: 30min, 60min, and 120min	mice	ICR	7 – 8 wks	female	56 (44 exposed and 12 controls)	healthy	20 ppm for 30 min	20 ppm for 60 min

Observations:

Injuries to the olfactory epithelium following exposure to SO_2 became more severe as duration of the exposure increased.

Edema, loss of cilia, epithelial thinning, and epithelial desquamation in the olfactory epithelium were observed in groups 2 (60 min) and 3 (120 min). There was no difference in histopathologic findings between group 1 and controls.

The basal lamina and the connective tissue were well preserved throughout the entire mucosa. Injuries to olfactory epithelium became severer with exposure time. These changes were further pronounced 24 h after exposure. Regenerated epithelia were not observed in any group. Scanning electron microscopic findings were consistent with light microscopic findings. Olfactory epithelial surface were consistent with light microscopic findings. Olfactory epithelial surface was sloughed off and revealed, underlining intact basal lamina.

As duration of exposure to SO2 increased, injuries to the olfactory epithelium became more severe. Injuries included nonspecific infiltration of inflammatory cells and loss of cilia with irregular epithelial surfaces. Also, epithelial thinning, focal necrosis or desquamation were observed.

There was no difference in histopathologic findings between group 1 and controls. Earliest histopathologic changes were seen in group 2. Most severe changes were 24 hours after exposure. In general, early changes in olfactory epithelium after exposure to SO2 were primarily degenerative rather than inflammatory.

There was no difference in histopathologic findings between group 1 and controls. The earliest histopathologic changes were observed in group 2 which consisted a widened intercellular space and thinning of the olfactory epithelium. The most severe changes were seen 24h after exposure where surface morphology of the cell was markedly altered.

*Statistically significant

Author's Conclusions:

The results of this study suggest that early lesions of olfactory epithelium after exposure to SO2 may be primarily degenerative.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	(-) female only Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level: if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? a) Specie (+) *Guideline: Rat, or justification for use of another mammalian specie.* b) Age (+) *Guideline: Adult, i.e.,8-12 weeks.* (-) female only) Sex d) Pre-test health (+) *Guideline: Females should be nulliparous and non-pregnant.* e) Weight variation (-) greater than 20% variation *Guideline: Below 20% variation* f) Source (no info) *Guideline: An established breeding facility or educational institution*

B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? ↳ *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? **(no info)** If exposure chambers were employed, were animals exposed during the equilibration period? ↳ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? **(no mention of temp and hum)**Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(-) **no info** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ↳ *Gvideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* Was the number of animals grouped in a single chamber stated? (+) ↳*Guideline: Groupings should permit clear observation of each animal.* Was the manner by which animals were grouped appropriate? (+) ↳*Guideline: Animals may be grouped by sex, or individually.* Was the type and source of feed and water stated? Was it appropriate? (no info) ↳ *Guideline: Conventional lab diet* Was the amount and feeding schedule specified?

	(no info) *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(+)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(-)** Was a full description of the monitoring devices provided? **(5 ppm)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	Was the acclimation period specified? Was it of acceptable duration? **(-) 3 days** *Guideline: 5 days* **(-)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(not stated)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(+)** Was a control group assayed? **(more detail needed)** Is the control regime appropriate? **(all animals sacrificed 72 h after exposure)** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	**(+)** Were appropriate endpoints examined? **(-)** Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(+)** Were accepted assessment methods employed? **(+)** Were assessment measures sufficiently sensitive? **(-)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(+)** Was the timing and frequency of assessment logical? **+)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(some)** Was individual data reported for each test animal?

G. Data analysis:	(no statistical analysis) Are the statistical methods used appropriate? (n/a) Are confidence intervals reported? (n/a) Did the investigator appropriately interpret the statistics? (n/a) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (more detail needed) Were assumptions reasonable? (-) Are the methods reproducible? (no info) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (no extrapolations made) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study suggested that early lesions induced by acute SO_2 exposure were primarily degenerative ones accompanying epithelial thinning and desquamation. It was not ascertained whether the difference in injury pattern between the olfactory and respiratory epithelia was due to innate resistance of the respiratory epithelium to SO_2 or other factors. The severity of lesions of the olfactory epithelium increased with exposure time.

This study was intended to analyze possible histopathological changes in the mouse epithelium after exposure to 20 ppm SO2. While respiratory-like epithelium was observed 72 hours after exposure to SO_2, such findings might be found normally in mice.

Confidence index:

There is no indication as to whether the animals were randomly assigned to the exposure or control groups.

There was no information provided as to the treatment of controls.

The mice used in this study were well-characterized according to age, gender, weight and pre-experimental health.

The weight variation was greater than 20%.

More detail is needed on the methods.

All of the mice were female.

There is no information on the housing and feeding.

Statistical data is lacking

Review – Confidence index ranking:

Low to moderate

NON-CLINICAL REVIEW FORM

Author: Corn, M., Kotsko, N., Stanton, D., Bell, W., Thomas, A.P., Pittsburgh, M.S.	**Internal ID:** 290

Title: Response of Cats to Inhaled Mixtures of SO_2 and SO_2 – NaCl Aerosol in Air

Year:1972

Abstract: Twenty healthy, adult male cats were lightly anesthetized with pentobarbital (Nembutal) sodium and tracheotomized; respiration was maintained by pump. Medical-grade breathing air, with or without sulfur dioxide alone or in combination with sodium chloride aerosol was delivered in predetermined exposure sequences. Pulmonary flow resistance and lung compliance were evaluated. Pollutants were also delivered via endotracheal catheter or face mask, or both. Approximately 20 ppm of SO_2 in air was required to evoke a significant change in pulmonary flow resistance in "reactors". The majority of animals showed no response to this concentration of SO_2 either alone or with NaCl aerosol (10 mg/m^3). An increased frequency of significant changes in pulmonary flow resistance was suggested when pollutants were delivered via endotracheal catheter or face mask. All alterations in parameters of response were reversible shortly after exposure ceased.

Objective: A series of investigations delineated the mechanisms of bronchoconstriction and peripheral airway constriction in cats. One reason for this study is to see whether there is a parallel between responses to SO_2, alone and in combination with sodium chloride aerosol, on pulmonary flow resistance and lung compliance reported in guinea pigs and those occurring in other species.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
Low SO_2:15-25 ppm High SO_2: 30-40ppm	One time; 30 minutes	cat	No info	Adult	male	20	healthy	No observed adverse effect level	Lowest observed adverse effect level

Observations:

Changes in Pulmonary Flow Resistance and Lung Compliance in Cats Following Pollutant Challenges

Approximately 20 ppm SO_2 in air is required before any animals show significant alterations in pulmonary flow resistance. At this concentration, only 2 animals in 20 responded. One animal in 20 showed a significant decrease in pulmonary flow resistance. The responses varied depending on the mode of administration. The results suggest that delivery of the pollutant via tracheal cannula did evoke fewer significant responses in pulmonary flow resistance and lung compliance. Small but insignificant changes in lung compliance were observed. Certain receptors were bypassed when pollutant was delivered by tracheal cannula suggesting that receptors in the nasopharyngeal chamber and proximal to the tracheal cannula can increase total lung resistance distal to the tracheal cannula.

The majority of animals showed no response to this concentration of SO_2 either alone or with NaCl aerosol.

Pathological Changes in Cats Following Pollutant Exposure
No changes detected between exposed and controlled animals.
*Statistically significant

Author's Conclusions:

"It was not possible to detect differences in airway caliber in thick and thin lung sections of control and exposed animals. Large changes in peripheral airway resistance could go undetected by measurement of R_L, which is insensitive to alterations in peripheral airway resistance. Our studies show that careful morphological examination of airway caliber is also insensitive to any changes that may occur in the ranges of R_L increases cited here."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	Is the number of animals sufficient to produce statistically significant results? **(no info on sex) (no info on number of animals per experiment)** *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? **(-) poor justification given** a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* b) Age **(no info)** *Guideline: Adult, i.e.,8-12 weeks.* **(no info)** c) Sex **(no specific info)** d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* **(>20%)**e) Weight variation *Guideline: Below 20% variation* **(no info)** f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? **(more info needed)** Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* **(more info needed)** Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of

	appropriate exposure levels? **(no info)** Were both nominal and actual exposure concentrations recorded? **(-)** Was the duration of exposure appropriate to investigate the primary objective? **(-)** Was the duration of exposure precisely defined? **(n/a)** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(-)** Was the mode of administration appropriate? **(more info needed)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(-)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? **(no info)** *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* Was the number of animals grouped in a single chamber stated? **(n/a)** *Guideline: Groupings should permit clear observation of each animal.* Was the manner by which animals were grouped appropriate? **(n/a)** *Guideline: Animals may be grouped by sex, or individually.* Was the type and source of feed and water stated? Was it appropriate? **(no info)** *Guideline: Conventional lab diet* Was the amount and feeding schedule specified? **(-)** *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(+)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)*

	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	Was the acclimation period specified? Was it of acceptable duration? **(-) no info** *Guideline: 5 days* **(-)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(not stated)** Were test animals randomly assigned to exposure groups? **(-)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(served as own controls)** Was a control group assayed? **(-)** Is the control regime appropriate? **(animals killed after exposure)** Was the period of observation following exposure appropriate?
F. Data collection:	**(+)** Were appropriate endpoints examined? **(-)** Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(more info needed)** Were accepted assessment methods employed? **(-)** Were assessment measures sufficiently sensitive? **(-)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(more info needed)** Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(some)** Was individual data reported for each test animal?
G. Data analysis:	**(more detail needed)** Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(-)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
H. Interpretations:	**(objective not clearly stated)** Was the original objective addressed? **(-)** Are the results novel? **(-)** Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable? **(-)** Are the methods reproducible?

	(-) Did either the study design or the statistical analysis limit confounding variables? **(more detail needed)** Do the conclusions make biological sense? **(-)** Are the extrapolations reasonable? **+)** Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Approximately 20 ppm of SO_2 in air was required to evoke a significant change in pulmonary flow resistance. The majority of animals showed no response implying that the concentrations were not appropriate for the study. The majority of animals showed no response to SO2, either alone or with NaCl aerosol. An increased frequency of significant changes in pulmonary flow resistance was suggested when pollutants were delivered via endotracheal catheter or face mask. However, none of the findings were of toxicological or clinical significance. The results were significantly variable between the cats.

Confidence index:

There is very limited information on the test animals.

The paper reports 29 cats and the abstract reports 20 cats.

For some experiments the sample size was as low as 3– it is not clear if results can be extrapolated to a larger population..

More detail is needed on the methods and procedures.

The use of cats is poorly justified.

The weight variation was greater than 20% and all of the cats were male.

None of the breathing was natural.

The presentation of the results is too brief.

More detail is needed on the control methods.

There is no information on whether the animals were randomly assigned.

The sequence of exposures varied for each cat. Cats exposed to the high concentrations first may have become more susceptible to further exposures than the animals that were first exposed to low concentrations.

Exposure time is not precisely defined.

The results are not adequately discussed.

There was large variability in responses of the cats.

The pathological analysis revealed no difference between cases and controls.

In the discussion, a control animal is defined as one previously exposed to SO_2 or SO_2-aerosol mixture in air. It is not clear then, what the difference between the controls and exposed are.

The responses differed depending on the mode of administration.

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM

Author: **Dalhamn, T., Strandberg, L.**

Internal ID: 294

Title: **Acute Effect of Sulphur Dioxide on the Rate of Ciliary Beat in the Trachea of Rabbit, In Vivo and In Vitro, with Studies on the Absorptional capacity of the Nasal Cavity**

Year: **1961**

Abstract: This article contains a detailed examination, of certain toxicologic problems regarding the acute effects of sulphur dioxide on the respiratory tract. Special interest has been devoted to the reactions of the ciliary epithelium, and to the absorption of sulphur dioxide in the respiratory tracts. The reactions of the ciliary epithelium have been observed both with the animals themselves inhaling the sulphur dioxide and with the gas being applied to the trachea in vitro.

Objective: To investigate the role of the polymorphonuclear leukocyte chemokines macrophage inflammatory protein 2 and KC. To study the absorption of sulphur dioxide in respiratory tracts to understand the toxicity of the gas.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
In vivo: 100, 200, 300ppm In vitro: 1. effect of varying SO_2 on the ciliary movement of trachea 4,5,6,7,9ppm 2. absorption of SO_2 in respiratory tract with exposure to ~100ppm 3. absorption of SO_2 in	In vivo – 45 min In vitro-45 min	rabbit	No info	No info	No info	In vivo: 10 animals for each exposure level In vitro: 1.varied for exposure 1-3 animals per level 2. 10 3. 10 4. 5 per exposure level	No info	In vivo: 100ppm In vitro: (exp 1) approx. 7ppm	

respiratory tract with exposure to ~250ppm 4. absorption of SO_2 in the nasal cavity with exposure to 100,200,300ppm 5. absorption of SO_2 in the mouth and pharynx at exposure to 200ppm						5. 5			

Observations:

In vivo:

In preliminary investigations, the results seem to suggest that no effect is to be expected for exposures under 100ppm, whereas exposures at 300 ppm and over always lead to the cessation of ciliary activity during exposure.

After 15 minutes of exposure the ciliary activity had stopped in 5 cases when the concentration was 200 ppm and in 8 cases at 300 ppm. With 100ppm no visible effect could be observed. After 30 min exposure no further changes had taken place, but after 45 minutes the ciliary activity stopped in a further case, both at 200 and 300 ppm. In the group exposed to 200ppm there remained only one animal with registrable ciliary activity in the 300 ppm group after 45 minutes exposure.

In the 100ppm group, the mean figure for the initial value was 1265 beats per minute; after 15 minutes, exposure was 1175 minutes, after 30 minutes, 1041 beats per minute, and after 45 minutes, 1091 beats per minute. If a statistical analysis is made, variation between the rabbits was 143 beats per minute and the error of method 33 beats per minute. The initial value in the 200ppm group was 1200 beats per minute and in the 300ppm group, 1077.

In vitro:

No effects could be observed from concentrations of less than around 7 ppm SO_2. With higher concentrations a cessation of ciliary activity was observed in all cases. The time when the effect took place varied within wide limits. The correlation between time of occurrence and SO_2 concentration was significant.

For the absorption studies, lower values for absorption were obtained with the continuous suction of air from the trachea via the nasal cavity and the relatively lowest values, finally, in those cases where the samples were taken continuously from the trachea via the cavity of the mouth. The concentration of SO_2in the trachea was around 5-10% of the original concentration, and no very manifest variation seems to have occurred during the 45 min in which the experiment was

taking place. During one of the exposures for 300ppm SO_2, an animal died about 10 min after the experiment had started.
*Statistically significant

Author's Conclusions:

"The experiments show that only a small part of the gas manages to penetrate down into the trachea via the absorption mechanism in the rabbit's nasal cavity which is extremely effective, at least in the case of sulphur dioxide. This is the case even with sulphur dioxide concentrations in the inhaled air many times greater than the contents recorded in smog. Even if absorption is different in man, as is probable, from that in rabbits, it still seems that the experiments made do not support the idea that the effects of smog are the result of an acute inhibition of the ciliary movements owing to a pure sulphur dioxide reaction."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	**(-) the sex of the animal is not given** Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (no info) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (no info) c) Sex d) Pre-test health (no info) *Guideline: Females should be nulliparous and non-pregnant.* e) Weight variation (no info) *Guideline: Below 20% variation* (no info) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	((-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of

	appropriate exposure levels? **(+)** Were both nominal and actual exposure concentrations recorded? **(+)** Was the duration of exposure appropriate to investigate the primary objective? **(unclear for some experiments)** Was the duration of exposure precisely defined? **(n/a)** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(+)** Was the mode of administration appropriate? **(+)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(monitored, but not clear if it was continuously)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(not stated)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(n/a)** Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* **(no info)** Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* **(not stated)** Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* **(no info)** Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(+)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined.

	Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water) **(+)** Was a full description of the monitoring devices provided? **(approx 5ppm)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(-)** Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* **(-)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(not stated)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(-)** Was a control group assayed? **(n/a)** Is the control regime appropriate? **(-)** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	**(+)** Were appropriate endpoints examined? **(-)** Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(+)** Were accepted assessment methods employed? **(+)** Were assessment measures sufficiently sensitive? **(-)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(+)** Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(+)** Was individual data reported for each test animal?
G. Data analysis:	**(no info)** Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(n/a)** Did the investigator appropriately interpret the statistics? **no info** Are the observed trends statistically significant?
H. Interpretations:	**(+)** Was the original objective addressed?

	(-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? **(more detail needed)** Were assumptions reasonable? (-) **more detail needed** Are the methods reproducible? (+) **some** Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The experiments n which the rabbits inhaled the sulphur dioxide show that no certain effect on the ciliary movement could be recorded at concentrations below about 200ppm. However, experiments which involved blowing the sulphur dioxide directly on to the trachea in vitro show that about 10 ppm of sulphur dioxide is enough to stop the ciliary movements. This may suggest that the nasal cavities may absorb large quantities of sulphur dioxide. It was concluded that it is unlikely that any acute inhibition of the ciliary movments takes place as an effect of SO2 alone.

This study attempted to detail the acute effects of sulphur dioxide. Previous studies have examined acute inhalation of SO_2 at lower concentrations. The authors point out that results with rabbits may vary considerably from rats since rats have a smaller trachea than rabbits.

Confidence index:

There is limited information on the test animals.

There is no indication as to whether the animals were randomly assigned.

The introduction is very detailed.

Some of the methods were very detailed, e.g. the monitoring of SO_2. Reliability of the method was tested.

Some confounders were considered.

The methods of exposure were well described.

Exposure time and concentration were not always clearly stated.

The inter and intra variability between rabbits was calculated.

The results were all presented in tables, but not well discussed in the paper.

This study did not detail any of the characteristics of the animals, including age, weight or gender. Details such as housing, feeding details, as well as acclimation period were not given and no control group was assayed. No statistical analysis was done and the methods would not be easily reproducible.

Review – Confidence index ranking:

Low to moderate

NON-CLINICAL REVIEW FORM

Author: Hanacek, J.	**Internal ID:** 300

Title:Influence of Sulphur Dioxide Breathing On Defensive Reflexes of the Airways

Year:1987

Abstract: The influence of sulphur dioxide (SO_2) on cough and expiratory reflexes was studied in 22 anaesthetized (pentobarbital, 30 mg/kg i.v.) rabbits. The cough reflex (CR) was elicited by tracheobronchial mucous membrane stimulation, using a soft venous catheter. In order to induce the expiratory reflex (ER) irritation of the larynx was performed by a silon fibre loop. The strength of both reflexes was assessed from the interpleural pressure fluctuation before SO_2 breathing, immediately after, and 30-120 minutes after SO_2 breathing had been stopped. The animals breathed SO_2 in 200-300 ppm concentration through a tracheal cannula. The influence of SO_2 on the direct dependence of lung inflation pressure magnitude (LIPM) on strength of the expiratory reflex (ERS) was tested. It was found that elicitability and strength of the cough reflex decreased immediately after SO_2 breathing had been stopped, and did not reach the preexposure level at the end of the experiment, i.e. 2 hours after discontinuation of SO_2 breathing. The direct dependence of LIPM on ERS in unaffected rabbits [10] was abolished immediately after SO_2 breathing had been stopped but was present again 30-60 minutes later, and did not differ significantly from the control values. The results give evidence of the strong depressive influence of SO_2 on the defensive reflexes of the airways in rabbits.

Objective: The purpose of the present paper was to determine the influence of short-term SO_2 breathing on the artificially induced cough reflex, and on the direct dependence of lung inflation pressure between the strength of the expiratory reflex.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
200-300ppm	10-15 minutes	Rabbits	New Zealand	Adult	Male and female	22	Unknown		

Observations:

SO_2 breathing caused decreases in both cough reflex elicitability and cough reflex strength in rabbits.

Elicitability of the cough reflex was decreased to 46% of control values immediately after SO_2 breathing and was restored 30 – 120 minutes later to 64% of the control. All quantitative cough parameters, except the number of cough efforts in one cough attack per minute, were significantly decreased immediately after SO2 breathing compared to counted values. The NE decreased to 20%, IME to 20-36%, IA to 6-10% of control values, immediately after SO_2 breathing had been stopped. The parameters mentioned did not reach the control value 30-120 min later.

All quantitative cough parameters except of NE per minute were significantly decreased immediately after SO_2 breathing compared to counted values.

The direct dependence of LIPM on ERS in unaffected rabbits was abolished immediately after SO_2 breathing had been stopped but was present again 30-60 minutes later, and did not differ significantly from the control values.

*Statistically significant

Author's Conclusions:

The results give evidence of the strong depressive influence of SO_2 on the defensive reflexes of the airways in rabbits.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	(+) **distribution of male and female is not given** Is the number of animals sufficient to produce statistically significant results? └ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie └ *Guideline: Rat, or justification for use of another mammalian specie.* (+) b) Age └ *Guideline: Adult, i.e.,8-12 weeks.* **(distribution not provided)** c) Sex **(no info)** d) Pre-test health └ *Guideline: Females should be nulliparous and non-pregnant.* **(>20%)** e) Weight variation └ *Guideline: Below 20% variation* **(no info)** f) Source └ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? └ *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective?

	(-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(+)** Were both nominal and actual exposure concentrations recorded? **(+)** Was the duration of exposure appropriate to investigate the primary objective? **(-)** Was the duration of exposure precisely defined? **(no chamber)** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(+)** Was the mode of administration appropriate? **(+)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(not stated)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(not stated)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(no info)** Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* **(no info)** Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* **(no info)** Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* **(no)** Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(-)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined.

	↳ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(-)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(no info)** Was the acclimation period specified? Was it of acceptable duration? ↳ *Guideline: 5 days* **(no)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(not stated)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(served as own controls)** Was a control group assayed? **(more info needed)** Is the control regime appropriate? **(-)** Was the period of observation following exposure appropriate? ↳ *Guideline: Longer than 14 days.*
F. Data collection:	**(+)** Were appropriate endpoints examined? **(-)** Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(more info needed)** Were accepted assessment methods employed? **(-)** Were assessment measures sufficiently sensitive? **(-)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(more info needed)** Was the timing and frequency of assessment logical? **(-)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each test animal?
G. Data analysis:	**(more info needed)** Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(more info needed)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
H. Interpretations:	**(+)** Was the original objective addressed?

	(-)Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (-) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study confirmed previous findings concerning the depressive influence of SO_2 on respiratory defensive reflexes and supposes that SO_2 may be one of the mechanisms participating in the formation and persistence of chronic bronchitis. However, the author agrees that SO_2 is only one of many other air pollutants that may produce this effect on the respiratory system.

Confidence index:

The results should be interpreted with caution due to a significant lack of information in the paper and weaknesses in the study design.

The introduction and literature review are inadequate.

The methods section lacks significant information and details.

There is limited information on the test animals including the distribution of males and females.

The weight varied up to 36%.

The cough reflex was induced by mechanical stimulation.

This study did not make mention of any of the experimental conditions including housing, feeding and acclimation prior to the experiment. The source of the rabbits is not mentioned, nor is their pre-experimental health. The sample size should have been larger to accommodate extrapolations of results to the population. A control group was utilized. An acceptable statistical analysis was used to produce some significant findings, however confidence intervals and raw data were not reported.

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM

Author: Baskurt, O.K., Levi, E., Andac, S.O., Caglayan, S.

Internal ID: 302

Title:Effect of Sulfur Dioxide Inhalation on Erythrocyte Deformability

Year:1990

Abstract: Erythrocyte deformability indexes of 14 male rats exposed to 1 ppm sulfur dioxide (SO_2) for 24 hours were compared with the values of a control group of 14 male rats. Deformability indexes were found to be increased, suggesting an impaired deformability, while the volume and hemoglobin contents of erythrocytes were unchanged. In a different set of control and experimental groups resealed erythrocyte membrane ghosts were prepared and the deformability, mean ghost volume and hemoglobin content were studied. No statistically significant differences were found for deformability and mean ghost volumes but the hemoglobin content of the ghosts prepared from the SO_2 treated rats were significantly higher than the control group value. It is postulated that, inhalation of SO_2 might impair the deformability of erythrocytes by increasing associations between hemoglobin and membrane proteins.

Objective: This study was performed to study the rheologic effects of SO_2 inhalation at erythrocyte level. Results were expected to provide some explanation for blood viscosity changes observed in a previous study.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
1ppm SO_2	24 hours	Rats	Swiss albino		Male	Erythrocyte deformability studies: 14 exposed and 14 control Resealed erythrocyte ghost studies: 11 exposed and 11 control	No info	No observed adverse effect level	Lowest observed adverse effect level

Observations:

No significant differences were found between the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values of control and SO_2treated groups. However erythrocyte deformability indexes of the SO_2 treated animals were found to be significantly higher than the control animals ($p<0.001$).

In resealed ghost studies, mean ghost volumes and ghost deformability indexes were not statistically different in control and SO_2treated groups. In the

SO_2treated group the mean ghost hemoglobin content was found to be significantly higher than the control group value ($p<0.05$).
*Statistically significant

Author's Conclusions:

It is postulated that, inhalation of SO_2 might impair the deformability of erythrocytes by increasing associations between hemoglobin and membrane proteins. "Mean cell volume and hemoglobin content was found to be unaffected by SO2 inhalation…membrane properties should be studied in order to explain the mechanism of SO2 effect on erythrocyte deformability. In this study, no significant difference was found between the resealed erythrocyte ghost deformability indexes of SO2 treated and control groups. In contrast, the mean hemoglobin content of resealed ghosts was found to be significantly higher in the SO2 treated animals. This suggests that more hemoglobin is being trapped in the erythrocyte ghosts of the rats exposed to SO2. This may provide indirect evidence for the increased associations between hemoglobin and membrane structures. Further studies are needed. Whatever the mechanism may be, it is demonstrated that SO2 inhalation may affect the rheologic properties of red cells. This change might be important for tissue perfusion, especially in microcirculation."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	**(+) but only male rats used** Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* **(no info)** b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) **male only** c) Sex **(no info)** d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* **(up to 60%)** e) Weight variation *Guideline: Below 20% variation* **(no info)** f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.*

	(more info needed) Were the chosen exposure levels appropriate to investigate the primary objective? **(-)** Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(+)** Were both nominal and actual exposure concentrations recorded? **(+)** Was the duration of exposure appropriate to investigate the primary objective? **(+)** Was the duration of exposure precisely defined? **(not stated)** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(+)** Was the mode of administration appropriate? **(+)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(not stated)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(not stated)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(-) not stated** Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* **(not stated)** Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* **(no info)** Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* **(no info)** Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(-)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined.

	↳ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(-)** Was a full description of the monitoring devices provided? **(no stated)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(-) not stated** Was the acclimation period specified? Was it of acceptable duration? ↳ *Guideline: 5 days* **(-)** Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? **(not stated)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) **(+)** Was a control group assayed? **(+)** Is the control regime appropriate? **(-)** Was the period of observation following exposure appropriate? ↳ *Guideline: Longer than 14 days.*
F. Data collection:	**(+)** Were appropriate endpoints examined? **(-)** Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(+)** Were accepted assessment methods employed? (more info needed) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(+)** Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(+)** Did the investigator appropriately interpret the statistics? **(-)** Are the observed trends statistically significant?
H. Interpretations:	**(+)** Was the original objective addressed? **(-)** Are the results novel?

	(-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? **(more info needed)** Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings suggest that SO_2exposure may affect the rheologic properties of red cells. However, further study is needed due to the weaknesses of this study and the lack of information presented. The authors extrapolate the findings to air pollution and cardiac mortality which has no basis from the results in this study. The purpose of this study was to explain blood viscosity changes observed in previous studies. Deformability of RBC could partially explain this viscosity and were found to be significantly higher in SO2-treated animals than control animals. Future studies should examine erythrocyte membrane properties to explain the mechanism of SO_2 effect on erythrocyte deformability.

Confidence index:

This study attempts to provide insight into the link between SO_2 and cardiac health. However, the validity of the results is jeopardized by a number of factors:
Confounding factors were not accounted for, such as age and pre-trial health.
There was limited information on the test animals.
The rats were all male and the weight variation was up to 60%.
There is limited information on the exposure conditions, i.e. equilibration periods.
No source of the rats is provided, nor are feeding, housing or watering details.
More detail is needed on the methods of blood analysis.
The introduction and literature review are inadequate.
The results section is too brief as is the discussion of the results.
A description of the reagents used in the trial was not provided and only one concentration of SO_2 was used.
However, the concentration was based on that used in a previous experiment.

Review – Confidence index ranking:

Low

CLINICAL REVIEW FORM

Author: Horstman, D., Roger, L.J., Kehrl, H., Hazucha, M., | **Internal ID:** 303

Title: Airway Sensitivity of Asthmatics to Sulfur Dioxide

Year:1986

Abstract: The purpose of this study was to describe for asthmatic subjects the distribution of individual bronchial sensitivity to sulfur dioxide (SO_2). Subjects were nonsmoking male asthmatics (n = 27) who were sensitive to inhaled methacholine. None of the subjects used corticosteroids or cromolyn sodium. Oral medications were withheld for 48 hr, inhaled medications for 12 hr prior to all testing. Each subject participated in four separate randomly ordered 10 min exposures to 0.00, 0.25, 0.50 and 1.00 ppm SO_2 at 26 degrees C, 70% relative humidity. During exposures, subjects breathed naturally and performed moderate exercise (VE, normalized for body surface area = 21 1/m2 X min). Before and 3 min after exposure, specific airway resistance (SRaw) was measured by body plethysmography. Those subjects whose SRaw was not doubled by exposure to 1.00 ppm were also exposed to 2.00 ppm SO_2. Dose response curves (relative change in SRaw, corrected for change in clean air vs SO_2 concentration) were constructed for each subject. Bronchial sensitivity to SO_2 [PC(SO_2)], defined as the concentration of SO_2 which provoked an increase in SRaw 100% greater than the response to clean air, was determined. Substantial variability in sensitivity was observed: for 23 subjects, PC(SO_2) ranged between 0.28 and 1.90 ppm, while for the remaining 4 subjects, it was greater than 2.00 ppm SO_2. The median PC(SO_2) was 0.75 ppm SO_2, and 6 subjects had a PC(SO_2) of less than 0.50 ppm. PC(SO_2) was not related (r = 0.31) to airway sensitivity to methacholine.

Objective: The purpose of this study was to characterize airway sensitivity to SO_2 for individual asthmatic subjects and to describe the distribution of individuals' airway sensitivity to SO_2 within a well-characterized group of relatively mild asthmatics.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0, 0.25,0.5,1.0, 2.0ppm	10 min for each exposure	Male	18-35y	27	Asthmatic; nonsmoker, nonsteriod user	Varied for subjects	Varied for subjects

Observations:

Substantial variability in sensitivity was observed: for 23 subjects, PC(SO_2) **(the concentration of SO_2 which provoked an increase in SRaw 100% greater than the response to clean air)** ranged between 0.28 and 1.90 ppm, while for the remaining 4 subjects, it was greater than 2.00 ppm SO_2. The median PC(SO_2) was 0.75 ppm SO_2, and 6 subjects had a PC(SO_2) of less than 0.50 ppm. PC(SO_2) was not related (r = 0.31) to airway sensitivity to methacholine.

*Statistically significant

Author's Conclusions:

"Results from the present study indicate that a well-characterized subpopulation of asthmatics exhibited a broad spectrum of sensitivities to SO2. Characterization of the distribution of individual responsiveness to SO2 within this group of mild asthmatics is pertinent to the assessment of the proportion of this population which might develop bronchoconstriction during ambient exposures to low concentrations of SO2."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(more info needed)** Is the number of subjects sufficient to produce statistically significant results? **(+) except work history** Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(-)** Are the subjects a representative sample of the population to which extrapolations will be made? **(no info)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? **(+)** Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	**(-)** Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? **(+)** Was there a gradient of exposure levels? **(+)** Were the chosen exposure levels appropriate to investigate the primary objective? **(-)** Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(+)** Were both nominal and actual exposure concentrations recorded? **(+)** Was the duration of exposure appropriate to investigate the primary objective? **(+)** Was the duration of exposure precisely defined? **(+)** Was the mode of administration appropriate? **(+)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(+) SO_2** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	**(-)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined **(-)** Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(+)** Were the subjects randomly assigned to exposure groups? **(no info)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(subjects served as own controls)** Was a control group assayed? **(+)** Is the control regime appropriate? **(+) double blind** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(-)** Was the length of follow-up appropriate?

	(+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? (+) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	**(limited statistical analysis)** Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(no info)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables? **(more info needed)** Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Results from this study indicate that a well-characterized subpopulation of asthmatics exhibit a broad spectrum of sensitivities to SO_2. The results were highly variable among the subjects indicating possible differences in individual sensitivities of the mechanism responsible for SO_2-induced bronchoconstriction or possible differences in the distribution and amount of SO_2 actually reaching sensitive sites in the airways. The subjects were quoted as being representative of young adults with "mild" asthma. However, the subjects were all male and there is no information on how they were selected.

Confidence index:

There is no information on the source and selection of the subjects.

The subjects were all male.

The methods were very detailed in explanation.

The method of analysis assumed that the magnitude of exercise induced bronchoconstriction is reproducible from day to day.

There was no evidence of the absence of an SO_2 exercise interaction.

This study was well designed and limited confounding factors by excluding asthmatics using steroids, those exposed to dusty environments and those who were not responsive to the methacholine challenge. A control group was employed.

Recruitment methods are not clear in this study and follow up was not done. Also, the number of subjects is not enough to produce statistically significant results. Finally, it is possible that the frequency of assessment at each exposure level lead to the variance in the results.

Review – Confidence index ranking:

Moderate

CLINICAL REVIEW FORM

Author: Linn, W.S., Shamoo, D.A., Spier, C.E., Valencia, L.M., Anzar, U.T., Venet T.G., Hackney, J.D.	**Internal ID:** 304
Title: Respiratory Effects of 0.75ppm Sulfur Dioxide in Exercising Asthmatics: Influence of Upper Respiratory Defenses	
Year:1983	
Abstract: To determine the influence of mouthpiece breathing on respiratory responses to sulfur dioxide (SO2), 23 young adult asthmatic volunteers were exposed in a chamber to 0.75 ppm SO2 during heavy exercise, once with breathing unencumbered and once while they wore noseclips and mouthpieces. These conditions (more severe than in typical ambient exposures) were deliberately chosen to produce significant physiological and clinical responses. Similar exposures to clean air served as controls. Exposure studies were separated by 1-week intervals and order was randomized. The protocol consisted of 10 min on a bicycle ergometer (mean load 650 kg-m/min, mean ventilation 40 liter/min), preceded and followed by response testing (body plethysmography, symptom questionnaires, and forced expiratory function tests; the last were performed only postexposure). During clean-air exposures, specific airway resistance (SRaw) and symptoms increased significantly, but no meaningful differences between mouthpiece breathing and unencumbered breathing were observed. Exposures to SO2 under these relatively severe conditions produced greater increases in SRaw than clean-air exposures regardless of the mode of breathing, but the excess increase was significantly greater with mouthpiece than with unencumbered breathing. Symptom changes and postexposure forced expiratory function showed qualitatively the same pattern of decrements with SO2 ad did SRaw, but the excess responses attributable to mouthpiece breathing did not attain statistical significance. Mouthpiece breathing can compromise upper-respiratory defenses against SO2 to the extent that responses are greater than with more natural breathing. The mode of breathing should be taken in account when applying laboratory human exposure data to air-quality risk assessment.	
Objective: A direct comparison of the effects of unencumbered breathing and mouthpiece breathing in the same volunteer subject. The primary hypothesis to be tested was that unencumbered breathing would produce a lesser response to SO_2 (relative to clean air) than mouthpiece breathing.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.75ppm	10 min	15 male 8 female	23±4 y	23	Nonsmoker, history of asthma		

Observations:

During clean-air exposures, specific airway resistance (SRaw) and symptoms increased significantly, but no meaningful differences between mouthpiece breathing and unencumbered breathing were observed. Exposures to SO_2 under these relatively severe conditions produced greater increases in SRaw than clean-air exposures regardless of the mode of breathing, but the excess increase was significantly greater with mouthpiece than with unencumbered breathing. Symptom changes and postexposure forced expiratory function showed qualitatively the same pattern of decrements with SO_2 as did SRaw, but the excess responses attributable to mouthpiece breathing did not attain statistical significance.
*Statistically significant

Author's Conclusions:

Mouthpiece breathing can compromise upper respiratory defenses against SO_2 to the extent that responses are greater than with more natural breathing. The mode of breathing should be taken in account when applying laboratory human exposure data to air-quality risk assessment.

"These results show that the bronchoconstrictive response to SO_2 exposure is likely to be less with unencumbered breathing than with breathing via mouthpiece. Our mouthpiece exposure data give an estimate of bronchoconstrictive response about 50% greater than that obtained from the unencumbered breathing data. Even with effective upper-respiratory defenses, many young adult asthmatics clearly can be expected to show clinically and physiologically significant responses to SO_2 at concentrations of 0.75ppm or less."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (+) except work history Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (+) but subjects were paid for participation Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?

D. Procedural:	. (+) Were the subjects randomly assigned to exposure groups? . **(no)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) . **(subjects served as own controls0** Was a control group assayed? . (+) Is the control regime appropriate? . **(double)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) . (+) Was the length of follow-up appropriate? . (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	. (+) Were appropriate endpoints examined? . (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) . (+) Were accepted assessment methods employed? . (+) Were assessment measures sufficiently sensitive? . (+) Was the nature and severity of symptoms graded against an explicitly defined scale? . **(no info)** Did qualified personnel conduct the exams? . (+) Was the timing and frequency of assessment logical? . (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? . **(some)** Was individual data reported for each subject? . (+) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	. **(+) but more detail needed** Are the statistical methods used appropriate? . (+) Are confidence intervals reported? . **(more detail needed)** Did the investigator appropriately interpret the statistics? . **(some)** Are the observed trends statistically significant?
G. Interpretations:	. (+) Was the original objective addressed? . (-) Are the results novel? . (+) Was the design of the study appropriate to investigate the primary objective? . (-) Were assumptions reasonable? . (-) Are the methods reproducible? . **(some)** Did either the study design or the statistical analysis limit confounding variables? . (+)Do the conclusions make biological sense? . (-) Are the extrapolations reasonable? . (+) Is the paper in a peer-reviewed journal?

	. (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

These results are not entirely novel, but reiterate previous findings that provided evidence that the bronchoconstriction response to SO_2 exposure is less with unencumbered breathing than with breathing via mouthpiece. The experimental conditions were not typical of ambient exposures. Exposures to SO_2 under these relatively severe conditions produced greater increases in SRaw than clean-air exposures regardless of the mode of breathing, but the excess increase was significantly greater with mouthpiece than with unencumbered breathing. The differences apply only to the hypothetical average asthmatic subject and are not necessarily generalizable to the population. In the study, a few individuals showed markedly greater excess responses to the mouthpiece while others showed only slight differences. Future studies should examine additional dose-response data to define the threshold of detectable effect and to determine how it relates to concentration, ventilation level and biological characteristics of the exposed individual.

Confidence index:

Details on exposure, SO_2 monitoring, and assessment techniques are included in another study.

Subjects were self selected. Ten subjects were recruited from a previous study. This study outlines characteristics of subjects including sex, age, height, health and age. Withdrawals and reasons for withdrawal are listed. While subjects were randomly assigned to experimental groups, it was not clear what the method of randomization was.

The subjects were required to take no meds for the week previous to exposure, but some may not have complied.

More detail is needed on the statistical analysis. Small sample size lead to results that are not statistically significant.

According to the authors, results of the four-way analysis of variance on the data were not straightforwardly interpretable since several significant multiway interactions were found.

The responses were variable and the standard deviations were quite high in some cases.

Blinding was considered "quasi-double" and a control group was identified.

Detailed description of the materials and apparatus used were not provided and there is a lack of raw data leading to an incomplete report. The sample size is not large enough to accurately extrapolate from.

Review – Confidence index ranking:

Moderate

NON-CLINICAL REVIEW FORM

Author: Stratmann, U., Lehmann, R.R., Steinbach, T., Wessling, G.	**Internal ID:** 305

Title:Effect of Sulfur Dioxide Inhalation on the Respiratory Tract of the Rat

Year:1991

Abstract: In order to study the initial effect of SO_2 exposure by electron microscopy, Wistar rats were subjected to the gas for 8 hours at a concentration of 800 ppm. A gradient of decreasing damage was observed in the tracheobronchial tree in peripheral direction. The trachea epithelium showed the most severe lesions represented by groups of detached cells, necrotic cells and disappearance of cilia and goblet cells. The entire apical surface was composed of short protrusions. A complementary ultracytochemical chromatin labelling method could demonstrate a gradient with a decreasing labelling rate of epithelial cells towards the peripheral airways. The cytochemical findings may indicate an increased mitotic and metabolic activity of epithelial cells surviving in a region of marked morphological damage as a response to high SO_2 concentration. This nuclear activity seems to decrease in peripheral parts of the respiratory tract along the gradient of decreasing morphological damage.

Objective: The purpose of this study was to examine the initial effects of a high SO_2 concentration and an acute exposure to the tracheobronchial tree of the rat on the ultrastructural level.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
800ppm	8hr	Rats	Gnotobiotic Wistar Rats	No info	male	5 exposed and 5 control	No info	n/a	n/a

Observations:

Ultrastructural findings of this study demonstrate a gradient of impairment within the different regions—the intensity of cellular impairment decreases with a longer distance from the source of SO_2 influx into the tracheobrochial tree. A gradient of decreasing damage showed the most severe lesions in the tracheal epithelium represented by groups of detached cells, necrotic cells and disappearance of cilia and goblet cells. The entire apical surface was composed of short protrusions. A complementary ultracytochemical chromatin labelling method could demonstrate a gradient with a decreasing labelling rate of epithelial cells towards the peripheral airways. This nuclear activity seems to decrease in peripheral parts of the respiratory tract along the gradient of decreasing morphological damage. There was disintegration of tracheal epithelium in region 1 and in region 2 and the following regions, there were slight signs of tissue destruction but many signs of structural alterations. The most striking alteration was firstly the complete loss of cilia and their replacement by short protrusions and secondly the disappearance of goblet cells. In animals that had inhaled SO_2, apical cytoplasm was found to form short, stump-like protrusions in both the former ciliated cells and goblet cells that showed neither cilia nor microvilli compared to the control animals. Mast cells were found in the vicinity of the leucocytes more frequently in the exposed animals than in the controls. Macrophages were founding the bronchial lumen.

Author's Conclusions:

The cytochemical findings may indicate an increased mitotic and metabolic activity of epithelial cells surviving in a region of marked morphological damage as a response to high SO_2 concentration. This nuclear activity seems to decrease in peripheral parts of the respiratory tract along the gradient of decreasing morphological damage. The ultrastructural findings of this study demonstrate a gradient of impairment within the different regions.

In our study the acute effect of SO_2 seems to be responsible for the disappearance of goblet cells.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	**(-) only male** Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* **(no info)** b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) **male** c) Sex **(no info)** d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* **(no info)** f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary

	objective? (+) Was the duration of exposure precisely defined? **(not stated)** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(+/-) no info on oxygen and temp was 26oC**Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(not stated)** Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* (no info) Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* **(no info)** Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* **(no info)** Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water* *may be withheld optionally.*
D. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-) Was a full description of the monitoring devices provided?

	(no info) What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(no info)** Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* **(no info)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(no info)** Were test animals randomly assigned to exposure groups? (n/a) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(more info needed)** Was the timing and frequency of assessment logical? +) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	**(n/a no statistical analysis)** Are the statistical methods used appropriate? (-) Are confidence intervals reported? **(n/a no statistical analysis)** Did the investigator appropriately interpret the statistics? (n/a) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary

	objective? **(more detail needed)** Were assumptions reasonable? **(-)** Are the methods reproducible? **(no info)** Did either the study design or the statistical analysis limit confounding variables? **(no clear conclusions)** Do the conclusions make biological sense? **(no extrapolations)** Are the extrapolations reasonable? **(+)** Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study introduces new findings on the effect of SO_2 on the permeability of airway epithelium, mucocilliary clearance rate and hematological parameters. In addition, this study details the initial effects after a short exposure to a relatively high concentration of SO_2 while most authors report on lower concentrations and longer exposure periods. Inhalation of SO_2 causes change at a nuclear level. This nuclear activity seems to decrease in peripheral parts of the respiratory tract along the gradient of decreasing morphological damage. The trachea epithelium showed the most severe lesions.

An 8hr exposure to 800ppm SO_2 resulted in a gradient of decreasing damage within the different regions studied. The concentration was quite high and was used to compare to studies that typically use lower concentrations and longer time periods. At this concentration, the acute effect was quite severe resulting in the complete loss of cilia and the replacement by short protrusions and secondly the disappearance of goblet cells. Details were provided on the type of damage but not the amount of damage and the sample size was very small.

Confidence index:

The introduction was very brief. The animals were sacrificed 40h after exposure, but justification was not given for this timing.

Experimental conditions such as temperature and humidity were controlled during the procedure however it is not clear whether animals were acclimated prior to the experiment. Pretesting conditions were not detailed.

There was no information on whether the animals were randomly assigned. There was no information on oxygen levels in the chamber.

The grouping of the animals was not stated While the care of the animals is alluded to in this study, no direct mention of is made of the feeding or housing employed. Information on monitoring devices was not provided. The rats were all male and source and age info are not provided.

There is no justification for the use of 800 ppm and there is no information on whether there was an equilibration period.

The results are novel and assumptions reasonable.

Review – Confidence index ranking:

Moderate

CLINICAL REVIEW FORM

Author: Schachter, E.N., Witek, T.J., Beck, G.J., Hosein, H.R., Colice G., Leaderer, B.P., Cain, W.	**Internal ID:** 306

Title: Airway Effects of Low Concentrations of Sulfur Dioxide: Dose-Response Characteristics

Year:1984

Abstract: The acute respiratory effects of exposure to low-level, short-term sulfur dioxide (SO_2) were studied in ten asthmatic and ten healthy subjects. Subjects were exposed in an environmental chamber in a double-blind, random sequence to SO_2) levels of 0.00, 0.25, 0.50, 0.75, and 1.00 ppm for 40 min. During the first 10 min subjects exercised on a cycloergometer at a level of 450 kpm/min. On separate days, subjects were exposed to 0 and 1.0 ppm SO_2 in the absence of exercise. In exercising asthmatic subjects, breathing 1 ppm SO_2 resulted in significant changes from baseline in airway resistance (Raw), forced expiratory volume in 1 sec (FEV1.0), maximal expiratory flow at 60% of the vital capacity (VC) below total lung capacity on the partial flow volume curve [MEF40% (P)] as well as reductions in flows at 50% of VC (Vmax50%). None of these parameters showed significant changes for exposures to 0.5 ppm or less, with the exception of small decreases (i.e., less than 0.2 L/sec) in Vmax50% at 0.25 and 0.5 ppm. Ten minutes after the end of exercise, there were no statistically significant differences from baseline, even though SO_2 was still present in the chamber atmosphere. For asthmatic subjects, the average changes in Raw, FEV1.0, MEF40%(P), and Vmax50% increased as SO_2 levels increased, suggestive of a dose-response relationship with a consistent effect first seen at 0.75 ppm. In individual exercising asthmatics, responses may occur at levels of SO_2 below 0.75 ppm. No changes were seen in healthy individuals on any day, or in asthmatic subjects at rest. These results indicate that asthmatic individuals engaged in moderate activity have transient bronchoconstricton following exercise in the presence of SO_2, with consistent changes first being noted for the group when ambient levels reach 0.75ppm.

Objective: We undertook a study of the airway responses to low levels of SO_2 by unencumbered breathing in asthmatic and nonasthmatic healthy individuals to examine the dose-response characteristics of this irritant gas.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Protocol 1 0,0.25,0.50,0.75,1.00ppm	40min	Asthmatics 5 men 5 women	26.1±6.3y	10 asthmatics and 10 normal	10 asthmatics and 10 healthy	No observed adverse effect level	Lowest observed adverse effect level
Protocol 2 0,1.0ppm	40min	Normal 4 men 6 women	27.3±5.1y				

Observations:

Measurements were taken to measure mean changes from baseline pulmonary function in these subjects following exercise and SO_2 inhalation. For the nonasthmatic subjects, no significant drops were seen in any of the pulmonary function parameters during either the exercise or nonexercise protocols at any of the SO_2 levels tested.

In exercising asthmatic subjects, breathing 1 ppm SO_2 resulted in significant changes from baseline in airway resistance (Raw), forced expiratory volume in 1 sec (FEV1.0), maximal expiratory flow at 60% of the vital capacity (VC) below total lung capacity on the partial flow volume curve [MEF40% (P)] as well as reductions in flows at 50% of VC (Vmax50%). None of these parameters showed significant changes for exposures to 0.5 ppm or less, with the exception of small decreases (i.e., less than 0.2 L/sec) in Vmax50% at 0.25 and 0.5 ppm. Ten minutes after the end of exercise, there were no statistically significant differences from baseline, even though SO_2 was still present in the chamber atmosphere. For asthmatic subjects, the average changes in Raw, FEV1.0, MEF40%(P), and Vmax50% increased as SO_2 levels increased, suggestive of a dose-response relationship with a consistent effect first seen at 0.75 ppm. In individual exercising asthmatics, responses may occur at levels of SO_2 below 0.75 ppm. Subjective lower respiratory symptoms in asthmatics appeared also to follow a dose-response relationship. For asthmatics, a general increase in lower respiratory symptoms was observed with increasing SO_2 concentrations whereas in healthy individuals, upper airway symptoms were more frequent.

*Statistically significant

Author's Conclusions:

"Our studies confirm the recent observations in asthmatics exposed to low level SO_2 during exercise, in that a dose-response relationship exists between the level of SO_2 and the airway response. Our study differs from previous observations in that the threshold dose level in earlier studies was, in general, lower and the degree of response to SO_2 greater…airway responses to SO_2 in asthmatics are dose-related and that the route of inhalation, the level of exercise, and possibly individual airway reactivity are important determinants of these effects."

"In conclusion, we suggest that airway responses to SO_2 in asthmatics are dose related and that the route of inhalation, the level of exercise, and possibly individual airway reactivity are important determinants of the severity of these effects."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed0 Is the number of subjects sufficient to produce statistically significant results? (+) except work history Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (+) no info on incentiveWas the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(+) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (not stated) Were previous preliminary trials conducted to identify the range of appropriate exposure levels?

	(+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+) Was a full description of the monitoring devices provided? **(not stated) but can detect a 0.05ppm difference**What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+)**double**Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(some)** Was individual data reported for each subject? (+) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics?

	(some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Normal individual subjects demonstrated no significant pulmonary function decrement at rest or during moderate exercise after inhalation exposure of up to 1 ppm SO_2. Asthmatics exposed to 1ppm SO_2 while at rest demonstrated no significant changes in lung function. When asthmatic subjects performed moderate exercise at the beginning of an exposure period, consistent decrements in most lung function occurred at 0.75-1.0ppm. These results suggest that asthmatics are more sensitive to low SO_2 levels and that the level of exercise and possibly individual susceptibility are important factors in determining the magnitude of the effects. There was limited information on symptoms to determine clinical significance.

This study determined that airway responses to SO_2 in asthmatics are dose-related. In this sense, these results are consistent with previous studies. However, results differ in that the threshold dose level in earlier studies was, in general, lower and the degree of response to SO_2 greater. Inconsistency with other studies is enhanced by a small sample size, making it unclear whether results can be extrapolated to a larger population.

Confidence index:

This study was generally well designed and the methods and exposure conditions were very detailed.

Consistency reported with some studies. Subjects were self-selected. There is no mention of an incentive.

Subjects were required to withhold meds and certain beverages before exposure days; however, some of the subjects may not have complied.

After the 30 min equilibration period in the chamber (subjects were in the chamber at this time), it took 5-10 minutes for SO_2 to reach the desired level. It is not mentioned if subjects were present during this 5-10 min period. There was variability in the individual responses. The sample size may have been too small.

This study employed a gradient of exposures in a double blind, random sequence. However, the method of randomization was not provided. Nonetheless, the subjects were thoroughly characterized and individual data was reported. The study clearly reported 0.75 ppm as the LOAEL.

Review – Confidence index ranking:

Moderate to low

CLINICAL REVIEW FORM

Author: Linn, W.S., Shamoo, D.A., Anderson, K.R., Whynot, J.D., Avol, E.L., Hackney, J.D.	**Internal ID:** 307

Title: Effects of Heat and Humidity on the Responses of Exercising Asthmatics to Sulfur Dioxide Exposure

Year: 1985

Abstract: Twenty-two asthmatic young adult volunteers, predetermined to be reactive to sulfur dioxide (SO_2) with exercise at normal room temperature, were studied to document short-term effects of SO_2 exposure under hot conditions, both humid and dry. For comparison, similar exposures were conducted at mild temperatures. All subjects were exposed in an environmental control chamber to all possible combinations of 2 atmospheric conditions (purified air and 0.6 ppm SO_2), 2 temperatures (near 21 and 38 degrees C), and 2 levels of relative humidity (near 20 and 80%). Exposures involved 5 min of heavy exercise (target ventilation rate, 50 L/min) plus brief warm-up and cool-down periods. Body plethysmographic measurements and symptom questionnaire interviews were administered before and at the end of each exposure. Response was expressed in terms of change in airway size or change in intensity of symptoms during exposure. Atmospheric condition showed the most marked and significant overall effect on physiologic responses; temperature and humidity effects were also significant. High temperature and high humidity tended to mitigate the bronchoconstriction produced by 0.6 ppm SO_2 exposure: group mean specific airway resistance approximately tripled at 21 degrees C and low humidity, but increased by less than 40% at 38 degrees C and high humidity. Temperature and humidity affected symptoms less consistently than physiologic responses, but in general, symptom responses paralleled physiologic responses.

Objective: To determine the effects of heat and humidity on the responses of exercising asthmatics to sulfur dioxide exposure.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.6ppm	Two approx. 5 min exposures (48h apart)/week for 4 weeks	9 female 13 male	18-33y	22	Asthmatics	No observed adverse effect level	Lowest observed adverse effect level

Observations:

Physiologic Results

Pre-exposure measurements of airway caliber showed significant variation related to temperature: resistance was higher at high temp.

In SO_2, mean SRaw increased approximately 35% (from 5.2 to 7.2 cm H_2O/s) at 38°C and high humidity, approximately 90% (from 5.0 to 9.4) at 38°C and low humidity, approximately 160% (from 4.2 to 10.8) at 21°C and low humidity. The corresponding decreases in men SGaw were 22, 44, 52 and 61% of the relevant preexposure means. Mean Vtg tended to increase with increased airway constriction. Most of the above results were not significant.

Symptoms

Temperature and humidity affected symptoms less consistently than physiologic responses, but in general, symptom responses paralleled physiologic responses.The most significant contributor to symptoms was SO_2 exposure. High temperature and high humidity tended to mitigate the bronchoconstriction produced by 0.6 ppm SO2 exposure: group mean specific airway resistance approximately tripled at 21 degrees C and low humidity, but increased by less than 40% at 38 degrees C and high humidity.

Atmospheric condition showed the most marked and significant overall effect on physiologic responses; temperature and humidity effects were also significant.

*Statistically significant

Author's Conclusions:

Temperature and humidity affected symptoms less consistently than physiologic responses, but in general, symptom responses paralleled physiologic responses.

The present results suggest that untoward responses to SO_2 are less likely in hot weather, either dry or humid, than at moderate temperatures.

"Increased temperature and humidity clearly mitigated the subjects' bronchoconstrictive response of these subjects to SO2 exposure during heavy exercise. From another viewpoint, if one takes the slight response under the hot, humid conditions as a reference point, it appears that a modest amount of airway cooling acts synergistically with SO2 exposure to induce bronchoconstriction during brief heavy exercise."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	. (more info needed) Is the number of subjects sufficient to produce statistically significant results? . (+) except work history Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? . (-) Are the subjects a representative sample of the population to which extrapolations will be made? . (+) no info on incentive Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? . (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	. (+) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? . (-) Was there a gradient of exposure levels? . (+) Were the chosen exposure levels appropriate to investigate the primary objective? . (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? . (+) Were both nominal and actual exposure concentrations recorded? . (+) Was the duration of exposure appropriate to investigate the primary objective? . (-) Was the duration of exposure precisely defined? . (+) Was the mode of administration appropriate? . (+) Was the frequency of administration appropriate to investigate the primary objective? . (+) Was evidence provided to justify the dosing regime?

	. W(+) ere exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	. (+) **details in another study** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined . (+) Was a full description of the monitoring devices provided? . **(not stated)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	. (+) Were the subjects randomly assigned to exposure groups? . **(no)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) . **(subjects served as own controls)** Was a control group assayed? . (+) Is the control regime appropriate? . (+) **double blind** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) . (+) Was the length of follow-up appropriate? . (+) **interview** Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	. (+) Were appropriate endpoints examined? . +) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) . (+) Were accepted assessment methods employed? . (+) Were assessment measures sufficiently sensitive? . (+) Was the nature and severity of symptoms graded against an explicitly defined scale? . **(*not stated)** Did qualified personnel conduct the exams? . (+) Was the timing and frequency of assessment logical? . **(no)** Was raw data provided to permit the reader to arrive at his/her own conclusions? . (-) Was individual data reported for each subject? . (+) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	. (+) Are the statistical methods used appropriate? . (+) Are confidence intervals reported? . **(more detail needed on interp)** Did the investigator appropriately interpret the statistics? . (-) Are the observed trends statistically significant?
G. Interpretations:	. (+) Was the original objective addressed? . (-) Are the results novel? . (+) Was the design of the study appropriate to investigate the primary objective?

	. (+) Were assumptions reasonable? . (+) Are the methods reproducible? . (some) Did either the study design or the statistical analysis limit confounding variables? . (+) Do the conclusions make biological sense? . (-) Are the extrapolations reasonable? . (+) Is the paper in a peer-reviewed journal? . (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings suggest that increased temperature and humidity mitigate the subjects' bronchoconstrictive response to SO_2exposure during heavy exercise. Health effects were less pronounced in warm weather, either dry or humid. The symptoms seemed to parallel the physiologic findings. However, the results were somewhat variable among the subjects and caution must be exercised in extrapolating the results to populations exposed to ambient air pollution. The subjects were pre-selected to be reactive to SO_2 so the greater unfavorable changes with SO_2 than with clean air are expected.

Confidence index:

The study was generally well designed. Aside from some variability in individual baseline values and responses, the study was generally well conducted.
The subjects were self-selected. Subjects were required to withhold meds during study period; however, this was not confirmed by testing.
Because this study dealt with the synergistic effect of SO_2 and relative humidity or temperature on an individual's health, the climatic conditions were always well documented. The number of subjects in the study may not have been high enough to extrapolate results to a similar population. However, the statistical methods used were valid, and produced statistically significant findings. Further studies might incorporate a control group.

Review – Confidence index ranking:

Moderate

NON-CLINICAL REVIEW FORM

Author: Knorst, M.M., Kienast, K., Muller-Quernheim, J., Ferlinz, R.	**Internal ID:** 308

Title: Effect of Sulfur Dioxide on Cytokine Production of Human Alveolar Macrophages in Vitro

Year:1996

Abstract: Tumor necrosis factor-alpha, interleukin-1beta, interleukin-6, and transforming growth factor-beta are cytokines synthesized by alveolar macrophages. We investigated the effect of sulfur dioxide, a major air pollutant, on the production of these cytokines by alveolar macrophages. The cells were layered on a polycarbonate membrane and exposed for 30 min to 0.0, 1.0, 2.5, and 5.0 ppm sulfur dioxide at 37 degrees C and 100% air humidity. The cells were incubated for 24 h after exposure, thus allowing cytokine release. Cytotoxic effects of sulfur dioxide were evaluated by trypan blue exclusion. Cytokines were measured with enzyme-linked immunosorbent assays (i.e., tumor necrosis factor-alpha, interleukin-1beta, and interleukin-6) or by use of a specific bioassay (i.e., transforming growth factor-beta). The toxicity of sulfur dioxide for alveolar macrophages ranged from 3.1 % to 9.5 %. A 30-min exposure to sulfur dioxide induced a significant decrease in spontaneous and lipopolysaccharide-stimulated tumor necrosis factor-alpha ($p < .001$) and lipopolysaccharide-stimulated interleukin-1beta release ($p < .05$). The release of interleukin-6 and transforming growth factor-beta was not affected significantly by sulfur dioxide exposure. Our results demonstrated a functional impairment of alveolar macrophages after sulfur dioxide exposure (i.e., release of tumor necrosis factor-alpha and interleukin-1beta). Neither spontaneous nor stimulated release of interleukin-6 and transforming growth factors were influenced by exposure to sulfur dioxide.

Objective: It was the purpose of the present in vitro study to investigate the effects of SO_2 on release of TNF-α, IL-1β, IL-6 and TGF-β by AMs (alveolar macrophages). TGF-β regulates the production of the extracellular matrix. Actions of the different cytokines are modified by the status of the target cells and by the concentration of synergistic and counteracting cytokines in the micromilieu of the lower respiratory tract. These mechanisms are targets of toxic air pollutants.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
0, 1.0, 2.5, 5.0 ppm	30 min	Human		56±5y	5 men 3 women	8	Bronchial carcinoma and who underwent fieroptic bronchoscopy for diagnostic purposes	No observed adverse effect level	Lowest observed adverse effect level

Observations:

The toxicity of SO_2 measured immediately after the 30 min exposure period, ranged from 3.1% (1 ppm) to 9.5% (5 ppm) ($p>0.05$). A reduction of 2% in cell viability was observed for the air exposed macrophages. No additional reduction in cell viability was recorded after 24 h of culture for both air exposed and SO_2 exposed macrophages. A 30-min exposure to sulfur dioxide induced a significant decrease in spontaneous and lipopolysaccharide-stimulated tumor necrosis factor-beta and lipopolysaccharide-stimulated interleukin-beta release. The release of interleukin-6 and transforming growth factor-beta was not affected significantly by sulfur dioxide exposure. Our results demonstrated a functional impairment of alveolar macrophages after sulfur dioxide exposure. Neither spontaneous nor stimulated release of interleukin-6 and transforming growth factor-beta were influenced by exposure to sulfur dioxide.

A dose-dependent decrease was observed in the spontaneous release of TNF-α after SO_2 exposure, highly significant at ($p<0.01$). The exposure to 2.5 ppm SO_2 resulted in a 32% decrease in mean stimulated TNF-α release ($p<0.01$).. Exposure to 5.0 ppm SO_2 did not induce a further decrease in mean TNF-α release ($p>.05$).

There were no significant differences in the spontaneous IL-1β release of AMs among the four exposure groups ($p>.05$).

For the LPS-stimulated IL-1β production of AMs, 30 min exposure to SO_2 resulted in a slight increase after exposure to 1ppm ($p>.05$) and in a subsequent significant decrease after exposure to 2.5ppm in LPS stimulated IL-1β release by AM. Exposure of the cells to 5ppm SO_2 did not result in a difference, compared with the control exposure ($p>.05$).

The SO_2 exposure of AMs yielded a small but nonsignificant reduction in spontaneous IL-6 production of AMs ($p>.05$) and in OPS-stimulated air ($p>.05$).

There were no significant differences in the spontaneous release of TGF-β between air and SO_2exposed cells ($p>.05$). Furthermore, a difference between spontaneous and LPS-stimulated TGF-β release of AMs was not observed.

No additional reduction in cell viability was recorded after 24h of culture for both air-exposed and SO_2-exposed macrophages.

*Statistically significant

Author's Conclusions:

"The results of the present study suggest that the damage to pulmonary tissue caused by So2 is not mediated by AM proinflammatory actions of TNF-α, IL-1β, IL-6 or by the mitogenic effect of TGF-β. Furthermore, our findings suggest that physical and functional damage to AMs occurs and is induced by short-term in vitro exposure to SO2, even below the threshold value established for SO2."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie (+) b) Age (+) c) Sex (+) d) Pre-test health (+) e) Weight variation

	(-) (f) Source
B. Exposure conditions:	(+) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (n/a) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(n/a) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? (n/a) Was the number of animals grouped in a single chamber stated? (n/a) Was the manner by which animals were grouped appropriate? (n/a) Was the type and source of feed and water stated? Was it appropriate? (n/a) Was the amount and feeding schedule specified?
D. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. (+) Was a full description of the monitoring devices provided?

	(not stated) What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(n/a)** Was the acclimation period specified? Was it of acceptable duration? **(n/a)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(n/a)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(n/a)** Was a control group assayed? (+) Is the control regime appropriate? **(n/a)** Was the period of observation following exposure appropriate?
F. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? **(n/a)** Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? **(more detail needed)** Were assumptions reasonable? (-) Are the methods reproducible?

	. (more detail needed) Did either the study design or the statistical analysis limit confounding variables? . (more detail needed) Do the conclusions make biological sense? . (-) Are the extrapolations reasonable? . (+) Is the paper in a peer-reviewed journal? . (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The results demonstrated a functional impairment of alveolar macrophages after sulfur dioxide exposure (i.e., release of tumor necrosis factor-alpha and interleukin-1beta). Neither spontaneous nor stimulated release of interleukin-6 and transforming growth factors were influenced by exposure to sulfur dioxide. The results provide further understanding of the role of AMs in the alveolar defense after exposure to irritant gases, however, more studies are needed to determine the clinical and toxicological significance of these results.

This study further elucidated the mechanisms involved in the harmful effects exerted by SO_2 on the respiratory tract. While previous studies have commented on the relationship between air pollution and cytokine release, conflicting results may be due to the limited information about interspecies variation of macrophage function in response to air pollution. Consequently, the use of the alveolar macrophage, such as in this study, serves as the most suitable cell for the study of the effects of SO_2 exposure.

Further experiments are required to elucidate why the ability of AMs to release TNF-α and IL-1β is impaired significantly after SO_2 exposure, where as their capacity to produce IL-6 and TGF-β is not affected.

Confidence index:

There were no similar studies for comparison.

The study was well designed and the methods section was very detailed.

This study utilized only 8 subjects, not adequate to extrapolate findings to a larger population. However, statistical analysis produced significant findings and the study was the first of its kind to report on the effect of SO_2 on the cytokine production of AMs. The author did not describe the recruitment process of the subjects, nor if they were properly informed of any experimental risks, however the health of the subjects was clearly reported, along with the subjects age, gender and the exposure period and gradient of exposures.

This was an in vitro study on human AMs; as such, study design, conduct, and reporting of the study is good, except that there is not evidence to justify a dosing regime nor were preliminary trials done to determine dose. Otherwise the data collection, statistical analysis, and interpretations are sound.

Review – Confidence index ranking:

Moderate to high

CLINICAL REVIEW FORM

Author: Linn, W.S., Avol, E.L., Peng, R.C., Shamoo, D.A., Hackney, J.D. | **Internal ID:** 309

Title: Replicated Dose-Resopnse Study of Sulfur Dioxide Effects in Normal, Atopic and Asthmatic Volunteers

Year:1987

Abstract: To help assess respiratory health risks from sulfur dioxide (SO_2) air pollution, we studied 24 normal, 21 atopic, 16 minimal/mild asthmatic, and 24 moderate/severe, medication-dependent asthmatic subjects classified according to history, lung function, allergy skin tests, serum IgE level, and airway reactivity to methacholine. All were exposed in a chamber (21 degrees C, 50% humidity) to 0.0, 0.2, 0.4, and 0.6 ppm SO_2 in random order at 1-wk intervals; then exposures were repeated to test consistency of response. The 1-h exposures included three 10-min exercise periods (ventilation approximately 40 L/min). Physiologic response was measured early (approximately 15 min) and late (approximately 55 min.) in exposure. Symptoms were evaluated during exposure and for 1 wk afterward. Normal and most atopic subjects showed little response at these SO_2 levels. A few atopic subjects and many asthmatics developed bronchoconstriction and respiratory symptoms, but most were able to maintain their exercise. Effects were not markedly different between early and late measurements, nor between the first and second round of studies; however, late and second-round responses appeared slightly more favorable. No statistically significant effect of SO_2 on symptoms was found 1 day or 1 wk after exposure. Minimal/mild asthmatics showed, on the average, slight responses at 0.0 ppm (attributable to exercise) and increasing responses at increasing SO_2 concentrations. Moderate/severe asthmatics reacted more at 0.0 ppm, but their increments in response with increasing SO_2 concentration were roughly similar to those of minimal/mild asthmatics. Thus, responses to SO_2 per se were not strongly dependent on clinical severity of asthma, nor on SO_2 exposure history during previous weeks.

Objective: To answer the following questions: 1. What range of physiologic and clinical response can be expected in diverse community populations exposed to ambient SO_2? 2. How does the intensity of response vary with clinical status? 3.. Is the response exacerbated or attenuated by repeated SO_2 exposures during a pollution season, i.e. is response affected by exposure history over periods of weeks? 4. To what extent do SO_2 associated symptoms and bronchoconstriction interfere wit normal activity?

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0 ,0.2, 0.4, 0.6 ppm	1 hour	Normal: 15M 9F Atopic:12M/9F Mild:10M/6F Moderate:10M/14F	18-37y	85 divided into 4 groups: Normal:24 Atopic:21 Mild Asthma:16 Moderate/Severe Asthma-24	See under number of subjects	No observed adverse effect level	Lowest observed adverse effect level

Observations:

Normal and most atopic subjects showed little response at the SO_2 levels investigated. A few atopic subjects and many asthmatics developed bronchoconstriction and respiratory symptoms, but most were able to maintain their exercise. Effects were not markedly different between early and late measurements, nor between the first and second round of studies. Late and second-round responses appeared slightly more favorable; however, the results were small and inconsistent. No

statistically significant effect of SO_2 on symptoms was found 1 day or 1 wk after exposure. Minimal/mild asthmatics showed, on the average, slight responses at 0.0 ppm (attributable to exercise) and increasing responses at increasing SO_2 concentrations. Moderate/severe asthmatics reacted more at 0.0 ppm, but their increments in response with increasing SO_2 concentration were roughly similar to those of minimal/mild asthmatics.
*Statistically significant

Author's Conclusions:

Responses to SO_2 per se were not strongly dependent on clinical severity of asthma, nor on SO2 exposure history during previous weeks.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (+) except work history Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (+) no incentive info givenWas the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(limited info) reference provided for more detailWas information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a fuli description of the monitoring devices provided?

	(not stated) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+) **double** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(few)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? **(more detail needed)** Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable?

	(+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Moderate/severe asthmatics showed the most unfavorable overall physiologic and symptomatic responses; however, responsiveness to SO_2 could not be predicted reliability by clinical status (as determined from history and screening tests) nor by responsiveness to exercise. There was variability among the individuals and some of the results were inconsistent.

Because volunteers were self-selected, it is unclear whether results could be extrapolated to a larger population. Nevertheless, results show agreement with a variety of earlier studies.

Confidence index:

The results of the experiments depended on the classification of the subjects into four groups. From observing some of the variability and inconsistency in responses, there was likely some misclassification.

Some subjects took meds during study.

The subjects were self selected.

Some of the baseline characteristics were considerably different among the subjects: a majority of moderate/severe asthmatics were women and men were in the majority otherwise. Five moderate/severe asthmatics were markedly obese. One moderate/severe asthmatic was 46 years old and 3 mod.severe asthmatics withdrew from the study.

This study employed a gradient of exposure levels and reported subject characteristics such as age and pre-trial health. Statistical analysis used was appropriate but did not produce statistically significant findings.

This report did not document any preliminary trials to determine appropriate exposure levels nor did it justify dosing. As well, there was no mention of monitoring devices.

Review – Confidence index ranking:

Moderate

D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+) **double** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)

E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (not stated) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (some) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (few) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (more detail needed) Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

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Confidence index:

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The subjects were self selected.

Some of the baseline characteristics were considerably different among the subjects: a majority of moderate/severe asthmatics were women and men were in the majority otherwise. Five moderate/severe asthmatics were markedly obese. One moderate/severe asthmatic was 46 years old and 3 mod.severe asthmatics withdrew from the study.

This study employed a gradient of exposure levels and reported subject characteristics such as age and pre-trial health. Statistical analysis used was appropriate but did not produce statistically significant findings.

This report did not document any preliminary trials to determine appropriate exposure levels nor did it justify dosing. As well, there was no mention of monitoring devices.

Review – Confidence index ranking:

Moderate

CLINICAL REVIEW FORM

Author: Linn, W.S., Venet, T.G., Shamoo, D.A., Valencia, L.M., Anzar, U.T., Spier, C.E., Hackney, J.D.	**Internal ID:** 310

Title:Respiratory Effects of Sulfur Dioxide in Heavily Exercising Asthmatics

Year:1983

Abstract: Twenty-three asthmatic volunteers 19 to 31 yr of age were exposed to 0 (control), 0.2, 0.4, and 0.6 ppm sulfur dioxide (SO_2) in random order at 1-wk intervals. Exposures took place in a controlled-environment chamber at 23 degrees C and 85% relative humidity; they included 5 min heavy exercise (mean minute volume, 48 L) plus time for postexercise physiologic testing. Body plethysmography (preexposure and end-exposure), spirometry (end-exposure only), and symptom questionnaires (covering the exposure period and the following week) all showed highly significant trends toward increased response with increasing SO_2 concentration. Pairwise statistical comparisons showed substantial, highly significant, changes at 0.6 ppm, relative to control. Fewer and smaller significant changes were found at 0.4 ppm. At 0.2 ppm, no significant physiologic changes were found, but increases in symptoms during exposure were possibly significant. Symptom reports 1 day and 1 wk postexposure showed no significant variation related to SO_2 level, i.e., exposure-related symptoms apparently reversed in less than 1 day.

Objective: We report here a further exploration of the respiratory effects of ambient-like SO_2 exposures in exercising asthmatics.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0, 0.2, 0.4, 0.6 ppm	5 min	10F 13M	19-31 y	23	asthmatic		

Observations:

The changes in SRaw during exposure showed a highly significant increase (pairwise statistical comparisons) in trend with increased SO_2concentraiton. In SO_2 exposures, increases relative to control were small and nonsignificant at 0.2ppm, somewhat larger and modestly significant at 0.4ppm, large and highly significant at 0.6ppm.

All measurements related to flow rates showed regular, highly significant, trends toward decrement with increasing SO_2 concentration. The FVC showed a qualitatively similar, but less pronounced and less highly significant trend. Comparisons of each SO_2 level with clean air showed no significant changes at 0.2 ppm. At 0.4 ppm only changes in Vmax at 75, 50, and 25% of FVC attained significance. At 0.6ppm, all forced expiratory changes were highly significant.

Symptom changes during exposures [body plethysmography (preexposure and end exposure), spirometry (end-exposure only), and symptom questionnaires (covering the exposure period and the following week)] showed a highly significant trend toward increased response with increasing SO_2 concentrations. The total symptom score change was significantly greater than the control value, even at 0.2ppm, although the level of significance was marginal.
Symptom reports 1 day and 1 week postexposure showed no significant variation related to SO2 level, ie exposure-related symptoms apparently reversed in less

than 1 day in most if not all subjects.
*Statistically significant

Author's Conclusions:

The measures of this study and closely related previous investigations give a reasonably clear and consistent picture of the response of exercising young adult asthmatics to brief SO_2 exposures concentrations attainable in ambient pollution episodes.

"Our findings, at least at 0.6 ppm, might be judged to show adversity, in that the subjects' sense of well being was clearly diminished, their degree of air-flow obstruction seemed sufficient to impair physical performance meaningfully, and drug treatment was clinically indicated in a few."

"Responses during oronasal breathing do indeed increase with dose rate, and show good agreement among different studies, despite differences in subjects and measurement conventions. Responses to forced oral breathing tend to be larger and less consistent than with oronasal breathing, but following a similar trend. Total dose is less satisfactory predictor of response than dose rate, as we observed little response in 1h exposures to 025 and 0.5 ppm, which produced a total dose considerably higher than any in the exposures. Furthermore, little excess response can be seen in the studies with a nominal exposure time of 10 min, compared with those with a nominal exposure time of 5 min."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(more info needed)** Is the number of subjects sufficient to produce statistically significant results? **(+) except work history** Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(-)** Are the subjects a representative sample of the population to which extrapolations will be made? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? **(not stated)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?

C. Equipment:	(+) as information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? **(no info)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(subjects served as own controls)** Was a control group assayed? (+) Is the control regime appropriate? (+) **double**Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(some)** Was individual data reported for each subject? (+)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible?

	(some) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:
Highly significant changes at 0.6 ppm, relative to control were observed . Fewer and smaller significant changes were found at 0.4 ppm. At 0.2 ppm, no significant physiologic changes were found, but increases in symptoms during exposure were possibly significant. Exposure-related symptoms apparently reversed in less than 1 day. The results were variable among the subjects. These findings relate to a small, self selected, volunteer population not necessarily representative of young asthmatics in general. The effects were qualitatively similar to the effects produced by exercise in clear air and didn't stop subjects from completing the exposure.
The exposure level was similar to that experienced in modern air pollution episodes.
Confidence index:
Study population was small and self selected.
The use of medications varied among the group.
All but one of the subjects were from previous studies.
Preexposure SRaw was not consistent among exposure conditions.
This study clearly characterized each subject, stating gender, age, height and weight. Each subject was its own control group and quasi-double binding was employed. While statistical analysis appeared to produce significant results, analysis was not described in this paper. Furthermore, sample size may have been inadequate to extrapolate results to a larger population.
The results from this study are similar to the results of previous studies. Results give a reasonably clear and consistent picture of responses of exercising young adult asthmatics to brief SO_2 exposures at concentrations attainable in ambient pollution episodes. Further studies should be done to include factors not present in lab studies but can be associated with SO_2.

Review – Confidence index ranking:

Moderate

CLINICAL REVIEW FORM

Author: Horstman, D.H., Seal, E., Folinsbee, L.J., Ives, P. Roger,L.J.,	**Internal ID:** 311

Title:The Relationship Between Exposure Duration and Sulfur Dioxide Induced Bronchoconstriction in Asthmatic Subjects

Year:1988

Abstract: The purpose of this study was to determine the shortest duration of exposure to 1.0 ppm sulfur dioxide (SO_2) sufficient to induce bronchoconstriction significantly greater than that observed with exposure to clean air (CA) in exercising SO_2-sensitive asthmatics. Asymptomatic, nonmedicated, male asthmatics (n = 12) with airway hyperresponsiveness to both methacholine and SO_2 were exposed in a chamber (20 degrees C, 40% relative humidity) for 0.0, 0.5, 1.0, 2.0 and 5.0 min to both CA and 1.0 ppm SO_2 on separate days (10 exposures). Just prior to each exposure, subjects walked on a treadmill in CA for 5 min at a predetermined speed/elevation to elicit a target ventilation of about 40 L/min, i.e., a brisk pace up a slight incline. After this walk, subjects rapidly entered an adjoining exposure chamber containing either CA or SO_2 and immediately walked at the same speed/elevation for the specified exposure duration. Subjects then rapidly exited the chamber. Specific airway resistance (SRaw) and ratings of respiratory symptoms associated with asthma [shortness of breath/chest discomfort (SB/CD) and wheezing (WHZ)] were measured prior to any exercise and following each exposure. Postexposure SRaw and symptom ratings increased with increased exposure duration in SO_2; postexposure SRaw also was increased with increased exposure duration in CA but to a lesser extent. After adjusting for the CA response, significantly greater SO_2-induced bronchoconstriction was observed for the 2.0 and 5.0 min exposures as indicated by substantially greater increases in SRaw and substantially higher ratings of respiratory symptoms. The authors conclude that with the above exposure conditions, on average, SO_2-sensitive asthmatics exhibit significant bronchoconstriction at exposure durations of 2.0 min or more.

Objective: The major objective of this study was to determine the minimal duration of exposure to 1ppm SO_2sufficient to elicit bronchoconstriction significantly greater than that observed with exposure to clean air (CA). A secondary objective was to describe the postexposure temporal pattern of SO_2induced bronchoconstriction.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1 ppm	0, 0.5, 1.0, 2.0, 5.0 min	Male	22-37yr	12	asthmatic		

Observations:

Postexposure SRaw and symptom ratings increased with increased exposure duration in SO_2; after adjusting for the clean air response, significantly greater SO_2-induced bronchoconstriction was observed for the 2.0 and 5.0 min exposures as indicated by substantially greater increases in SRaw and substantially higher ratings of respiratory symptoms. Significant differences ($p<0.025$) in the change in SRaw between clean air and SO_2 occurred for the 2min (9.2cm H_2O.s) and the 5min (20.3cm H_2Os) exposure durations. The recovery period was highly variable among the subjects.

There was no difference in group mean preexposure Sraw across exposure conditions. The individual subject's coefficient of variation ranged from 8.4% to 39.6%, indicating that the status of the subjects' airways was similar prior to exposure under all conditions.

In CA mean ratings of both shortness of breath/chest discomfort (SB/CD) and wheezing were consistently low across all exposure durations. With increasing duration of SO_2 exposure, the mean rating of SB/CD increased from 0.6 to 2.6 while that for wheezing increased from 0.3 to 2.6.

*Statistically significant

Author's Conclusions:

The authors conclude that with the above exposure conditions , on average, SO_2 sensitive asthmatics exhibit significant bronchoconstriction at exposure durations of 2 min or more.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+) but no work history Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (+) no info on incentiveWas the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-)Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+)Was evidence provided to justify the dosing regime? (+)Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+)Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+)Was a full description of the monitoring devices provided? 0.02ppm) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (not stated) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random

	assignment) **(subjects served as own controls)** Was a control group assayed? (+) Is the control regime appropriate? (+) **blinding impossible as subjects reported smelling the SO2** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Were appropriate endpoints examined? (+)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (some) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? (+) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? **(more detail needed)** Were assumptions reasonable? (-) Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The authors observed that 2 min was the shortest duration of exposure to 1 ppm SO_2 for which significant bronchoconstriction occurred in asthmatics breathing unencumbered. Care must be taken when extrapolating these findings to the general population of asthmatics since some asthmatics, who otherwise meet the criteria of asthma do not demonstrate SO_1 responsiveness. The magnitude of SRaw increases were high and the sample size may have been too small to detect significance. These results are relevant in that wind downdrafts due to building wake often increase ambient SO2 concentrations to relatively high levels in areas close to emission sources. Future studies should focus on the time course of recovery and its relationship to the peak response postexposure.

Confidence index:

Methods section is very detailed.

Subjects were instructed to withhold meds. However, this wasn't confirmed.

Most of the responses of the individual subjects did not show much variation; however, the post exposure recovery was highly variable and the results were inconsistent.

Results from this study may not be statistically significant based on the small number of subjects involved. Furthermore, confidence intervals are not reported making the statistical analysis questionable. The subjects were self selected.

The authors characterized each subject and limited confounding factors by using only male subjects. Each subject served as their own control and exposure durations were individually randomized for each subject.

The subjects in the study are only representative of the 19-30 year age group.

In addition, it is questionable whether results can be extrapolated to a more general population.

Review – Confidence index ranking:

Moderate

CLINICAL REVIEW FORM – non-clinical humans

Author: Kienast, K., Muller-Quernheim, J., Knorst, M., Lubjuhn, S., Ferlinz, R.	**Internal ID:** 312
Title:In Vitro Study of Human Alveolar Macrophage and Periopheral Blood Mononuclear Cell Reactive Oxygen-Intermediates Release Induced by Sulfur Dioxide at Different Concentrations	
Year:1994	
Abstract: Sulfur dioxide (SO_2) is a major air pollutant in urban areas. Alveolar macrophages (AM) located on the alveolar surface are in direct contact with this inhaled gas. We evaluated the dose-dependent effect of SO_2 exposure on the oxidative metabolism of AM and peripheral blood mononuclear cells (PBMNC) by measuring the spontaneous and stimulated reactive oxygen intermediates (ROI) release. AM or PBMNC were placed on a polycarbonate membrane, which was in direct contact with the surface of a nutrient reservoir. For exposure of the cells to SO_2 a special chamber was employed, in which humidified standard air with 5% CO_2 at 37 degrees C was mixed with SO_2 at the desired concentration. Periods of time between 30 and 120 minutes and concentrations between 0.3 and 1.5 ppm SO_2 were chosen for exposure. Thirty minutes exposure of AM to SO_2 (0.3-1.5 ppm) yielded a dose-dependent stimulation of ROI release; 2.0- to 3.6-fold of control ($r = 0.965$, $p < 0.005$). An exposure of 120 minutes to SO_2 resulted in a similar ROI production of about 2.5-fold at all tested concentrations. These experiments provide evidence that AM and PBMNC become activated by SO_2 producing large amounts of ROI.	
Objective: It was the aim of the present study to investigate if SO_2 is capable of inducing reactive oxygen intermediates release by alveolar macrophages.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.3-1.5ppm	30-120 minutes	13 males 1 female	Mean age 58±16 years	14	Bronchial carcinoma	n/a	n/a

Observations:

Periods of time between 30 and 120 minutes and concentrations between 0.3 and 1.5 ppm SO_2 were chosen for exposure. At 30 minutes exposure time a maximal increase in ROI release was measured for 1.5ppm SO_2.Thirty minutes exposure of AM to SO_2 (0.3-1.5 ppm) yielded a dose-dependent stimulation of ROI release 2.0- to 3.6-fold of control ($r = 0.965$, $p < 0.005$). Prolonged exposure times of up to 60 minutes also induced dose-dependent ROI production, although there were only insignificant differences, compared to ROI releases induced at the different SO_2 concentrations. An exposure of 120 minutes to SO_2 resulted in a similar ROI production of about 2.5-fold at all tested concentrations. Using PBMNC, similar reactions were observed.

The maximum ROI release for all exposure times was observed after 30 minutes exposure to 1.5ppm SO_2.

*Statistically significant

Author's Conclusions:

"Short time SO_2 exposure resulted in a does-dependent triggering of the NADPH oxidase system. After prolonged exposure periods, however, we recorded a similar response to SO_2 at all concentrations tested and an uninjured cell viability. This phenomenon could be explained by a maximal stimulation of the cellular activation mechanisms employed by SO_2. The slight decrease of ROI production after prolonged exposure might be due to an exhaustion of the NADPH oxidase system."

"In summary, SO_2 exposure causes a significant increase in ROI production, leading to the hypothesis that inflammatory pathomechanisms are influenced by SO_2-elicited ROI release."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]
This is an invitro study, so the questions were answered accordingly.

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (no info) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (n/a invitro assay) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (n/a) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? (n/a) invitro study Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(n/a invitro study) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (n/a) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(n/a) Were the subjects randomly assigned to exposure groups?

	(n/a) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (+) **control experiments** Was a control group assayed? (+) Is the control regime appropriate? **(n/a) invitro study** Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a invitro study)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(-)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each subject? **(n/a)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? **(-)** Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? **(-)** Are the methods reproducible? **(more info needed)** Did either the study design or the statistical analysis limit confounding variables? **(n/a)** Do the conclusions make biological sense? **(no extrapolations made)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

In this study, the exposure of AM and PBMNC cells to SO_2 induced cell activation and subsequent ROI production. However, as reported by the authors, there is no conclusive evidence as to the measured amount of ROI that is sufficient for inducing clinically relevant pulmonary fibrosis.

This study indicated that SO_2 exposure causes a significant increase in ROI production, suggesting that inflammation pathomechanisms are influenced by SO_2 elicited ROI release. However, because a small number of subjects were employed in this study, future studies should consider using a larger sample size to ensure that results can be extrapolated to the population.

SO_2 inhalation causes a significant increase in ROI production leading to the hypothesis that inflammatory pathomechanisms are influenced by SO_2-elicited ROI release.

Confidence index:

This study was generally well designed and informative.

Thirteen of the 14 patients were male.

The methods were tested and validated.

Control experiments were included.

This study was designed to evaluate the occupational exposure not environmental exposure.

The authors did not attempt to make any unreasonable conclusions based on the results.

This study attempted to limit confounding factors by restricting the study to smokers within a specific age range. However, no mention of recruitment techniques were given nor were experimental risks communicated to the subjects. It is not clear if randomization was used and if so, which method.

This study fails to be specific on the equipment used, and it fails to mention confidence intervals. Because it is an in vitro study, a lot of the housing/feeding requirements were not applicable. Exposure conditions were not mentioned.

Review – Confidence index ranking:

Moderate to low

CLINICAL REVIEW FORM

Author: Linn, W.S., Shamoo, D.A., Venet, T.G., Bailey, R.M., Wightman, L.H., Hackney, J.D.	**Internal ID:** 313

Title: Comparative Effects of Sulfur Dioxide Exposures at 5°C and 22°C in Exercising Asthmatics

Year: 1984

Abstract: Either airway cooling or sulfur dioxide (SO_2) can induce bronchoconstriction in many asthmatics. Whether these two stresses act synergistically is a question with important public health implications. Eight young adult asthmatic volunteers were exposed to SO_2 at 0.0, 0.2, 0.4, and 0.6 ppm, during 5 min heavy exercise at 5 degrees C, both with high (approximately 85%) and with low (approximately 50%) relative humidity. Physiologic response increased with increasing SO_2 concentration but did not vary significantly with humidity. Symptom response was marginally greater at low than at high humidity. Twenty-four asthmatics were exposed similarly to clean air and to 0.6 ppm SO_2, at 5 degrees C and also at 22 degrees C, always at high relative humidity. For this group, physiologic and clinical responses to SO_2 (in excess of responses to clean air) were highly significant, regardless of temperature. The mean excess responses at 5 degrees compared with those at 22 degrees C were not statistically significant in clean air or SO_2. Thus, moderate cold stress exacerbated the untoward response to SO_2 only slightly and inconsistently in these asthmatic subjects.

Objective: The present study tested the effect of cold on SO_2-induced bronchoconstriction under conditions milder than in the study by Bethel and coworkers (7). To determine the physiological effects of sulfur dioxide exposures at 5°C and 22°C in exercising asthmatics.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Experiment 1: 0.0, 0.2, 0.4, 0.6 ppm SO_2 Experiment 2: 0.6 ppm SO_2	Experiment 1: 10 minutes Experiment 2: 5 minutes	7 female 19 male	18-31 y	Experiment 1: 8 Experiment 2: 24	Clinically mild to moderate asthma		0.6ppm at 22°C

Observations:

Experiment 1:
All measures of response showed increasing tendencies to dysfunction with increasing SO_2 concentrations. The effect, as revealed by the interaction of SO_2 concentration and time (preexposure versus postexposure), was significant ($p<0.05$) for all measures except those of SRaw.
Physiological response increased with increasing SO_2 concentrations but did not vary significantly with humidity. Symptom response was marginally greater at low than at high humidity.

Experiment 2:
Physiologic and clinical responses to SO_2 (in excess of responses to clean air) were highly significant, regardless of temperature. The significant ($p<0.001$) SO_2 time interactions reflected consistently larger increases in SRaw and symptoms preexposure to post exposure in SO_2 than in clean air. The overall differences

between preexposure and postexposure measurements (main effects of time) were also highly significant as were the overall differences between clean and SO_2 days (main effects of SO_2) Furthermore, upper respiratory symptoms increased significantly more during SO_2 exposures than during clean air exposures ($p<0.003$ for SO_2 time interaction). The mean excess responses at 5 degrees compared with those at 22 degrees C were not statistically significant in clean air or SO_2. Thus, moderate cold stress exacerbated the untoward response to SO_2 only slightly and inconsistently in these asthmatic subjects.

*Statistically significant

Author's Conclusions:

Moderate cold stress exacerbated the untoward response to SO_2 only slightly and inconsistently in these asthmatic subjects.
At 5C, relative humidity variations over the common meteorologic range are not likely to have a great influence on physiologic response to brief heavy exercise, with or without SO_2 exposure.
"The results of Experiment 2 indicate only a small and inconsistent excess effect of SO_2 at 5C compared with that at 22C, whether measured in terms of airway caliber or symptoms. At least 3 interpretations are possible: (1) Cold has little effect on the responses to SO_2 of most exercising asthmatics. (2) Cold increases the responses to SO_2 of most exercising asthmatics; the typical effect is slight at 5C but more pronounced at colder temperatures. (3) Cold and SO_2 interact differently in different persons—synergistically in some, indifferently in others, antagonistically in still others."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(more info needed)** Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? **(more info needed)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? **(duration unclear in Experiment 2)** Was the duration of exposure appropriate to investigate the primary objective? (-) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? (+) **but not clear if it was continuous** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?

C. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided? **(not provided)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) (-) Was a control group assayed? (+) **group served as own controls** Is the control regime appropriate? **(single and double in some cases)** Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+)Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(more info needed)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible?

	(some) Did either the study design or the statistical analysis limit confounding variables? (no clear conclusions made) Do the conclusions make biological sense? (no extrapolations) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The results from experiment 1 suggest that at 5°C relative humidity variations over the common meteorological range are not likely to have a great influence on physiologic response to brief heavy exercise with or without SO_2 exposure. These results should be interpreted with caution, given the small sample size and nonideal experimental design. The results of experiment 2 indicate only a small and inconsistent (i.e. non statistically significant) excess effect of SO_2 at 5°C compared with that at 22°C, whether measured in terms of airway caliber or symptoms.

To remove uncertainties, additional studies combining subfreezing temperatures and realistic exposure conditions would be useful.

Confidence index:

The sample size for Experiment 1 was small, n=8.

There was single, and in some cases double blinding.

Exposures were separated by 7 days in experiment 2 and occurred in random order.

There is insufficient detail on the selection of subjects for this study.

Diagnosis of asthma was verified by physician.

Though subjects were instructed to withhold medication on days of the study, some admitted to using medication during study.

There is good detail provided on the characteristics of the subjects.

There was a significant amount of variability between the subjects in Experiment 2, making the results unreliable. The individual responses did not show a corresponding degree of consistency over time.

This study utilized multi-way analysis of variance to interpret findings. To minimize the opportunity for type I errors, Greenhouse-Geisser adjustments were applied. Statistical analysis produced some significant results that could be extrapolated to a similar population.

More studies (possibly with time as a manipulated variable) need to be done to resolve the inconsistencies and to come up with new ideas.

Review – Confidence index ranking:

Low to moderate

CLINICAL REVIEW FORM

Author: Linn, W.S., Shamoo, S.A., Vinet, T.G., Spier, C.E., Valencia, L.M., Anzar, U.T., Hackney, J.D.	**Internal ID:** 314
Title: Combined effect of sulfur dioxide and cold in exercising asthmatics	
Year:1984	
Abstract: Twenty-four asthmatic volunteers were exposed to 0, 0.3, and 0.6 ppm sulfur dioxide (SO_2) in purified background air at each of three temperatures--21 degrees, 7 degrees, and -6 degrees C--in a controlled-environment chamber. Relative humidity was approximately 80%. Exposures consisted of 5 min heavy exercise periods plus brief warmup and cooldown periods. Airway resistance, thoracic gas volume, and symptoms were measured immediately before and after exposure. For the group, increasing SO_2 concentration and decreasing temperature were associated with statistically significant unfavorable effects on airway resistance and respiratory symptoms, as expected from previous findings. Effects of SO_2 and cold usually appeared to combine in an additive or less-than-additive fashion; there was little evidence of synergism. Individuals' response patterns were variable: a few suggested synergism, but others suggested a mitigating effect of cold on the bronchoconstrictive response to SO_2.	
Objective: We have conducted exposure studies in a controlled-environment chamber to investigate whether cold/SO_2 synergism occurs under a closer approximation to "real-life" conditions.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0ppm, 0.3ppm, 0.6ppm	Approx 5 minutes including brief warm up and cool down periods.	11 female 13 male	19-33y	24	asthmatics		

Observations:

Pre- to post exposure changes of mean SRaw showed an approximately additive pattern, for increasing SO_2 and decreasing temperature, as indicated by the nonsignificant p value for interaction and the roughly parallel SO_2 dose-response curves for different temperatures. The Vtg changes showed a similar pattern.

The mean Sgaw changes behaved differently. The unfavorable effect of temperature was less consistent (less highly significant) than in the case of SRaw. The interaction between SO_2 and temperature factors was significant and indicated that their effect combined in a less than additive fashion. In clean air, the negative effect of decreasing temperature on mean Sgaw was consistent and pronounced; but in 0.6ppm SO_2, the mean Sgaw responses varied only slightly an inconsistently with temperature.

All exposure conditions increased the number and/or severity of symptoms reported. Lower-respiratory symptoms dominated the responses. Their statistical results suggested the SO_2 and cold acted more or less additively increasing the response, i.e., main effects of both stresses were significant but the interaction between them was not. The effect of temperature was less highly significant than that of SO: low temperature added less to the score change at 0.6ppm than at the lower SO_2 concentrations. Nonrespiratory symptom changes did not vary significantly with SO_2 concentrations or temperature. Then the total symptom score change with exposure was considered, the effect of SO_2remained highly significant. In the 24 hr periods post exposure, symptom scores were generally similar to the corresponding pre-exposure values, and showed no significant variation related to SO_2 or temperature, indicating that exposure effects reversed quickly after exposure ceased. D

For asthma symptoms, the SO_2 dose response curves had more positive slopes at -6°C and 7°C than at 21°C. That is, the average subjects' rating of the overall severity of asthma symptoms increase more, for a given increase in SO_2 exposure, at low temperatures than at 21°C. However, the differences were modest, and most subjects rated their overall asthmatic symptoms as mild even at -6°C and 0.6ppm.

Effects of SO_2 and cold usually appeared to combine in an additive or less-than-additive fashion; there was little evidence of synergism.

Individuals' response patterns were variable: a few suggested synergism, but others suggested a mitigating effect of cold on the bronchoconstrictive response to SO_2.

*Statistically significant

Author's Conclusions:

Overall, the present subjects' responses to cold and SO_2 separately seem consistent with expectations based on past studies, thus it seems reasonable to expect that their range of responses to both stresses together should be typical of young, mild-to-moderate asthmatics.

"The aggregate results of this study—clinical as well as physiological—do not support the hypothesis that SO2 and cold act synergistically in constricting the airways of exercising asthmatics. Cold undoubtedly did exacerbate the overall response to SO2, but in the typical subject, the combined stresses acted additively at most. Atypical individual subjects—those with apparently synergistic responses and those with apparently antagonistic responses—may represent distinct subgroups of the asthmatic population with as-yet-unidentified biological characteristics which modify their reactivity."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made?

	(-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) no info on incentive Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(-) reference to details in another study Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (-) Was a control group assayed? (n/a) Is the control regime appropriate? (+) double Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive?

	(+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? -) Was raw data provided to permit the reader to arrive at his/her own conclusions? **(some)** Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? **(no info)** Did either the study design or the statistical analysis limit confounding variables? **(n/a)** Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study looked at the combined effects of temperature and SO_2. Cold did exacerbate the overall response to SO_2, but in the typical subject the combined stresses acted additively at most, not synergistically. However, there was considerable variability between the individuals leading to inconsistent results. Atypical subjects, those with synergistic results, may represent distinct subgroups of the asthmatic population or the result of chance.

This study was variable in its results. Some data suggested a mitigating effect of coid on the bronchoconstrictive response to SO_2.. However, since this study involved a small, self-selected group, caution should be exercised in extrapolating results.

As expected, severity of symptoms increased with exposure concentration. Results from this study are consistent with past findings. Caution is necessary in applying the results to community air pollution exposure situations, which may involve additional environmental stresses not present in laboratory exposures.

Confidence index:

Interindividual variability was present, resulting in inconsistent results. Some of the results were additive and in some subjects the results were synergistic. The sample population was small and self-selected. Some of the subjects were recruited from a previous similar study which may have resulted in bias.

Good detail in the methods section, except re. the monitoring and exposure chamber. References are given to previous studies.

This study incorporates a gradient of exposure levels in a controlled-environment chamber. The study described volunteer recruitment and listed subject characteristics such as sex, age, weight, height and work load. The statistical method used was appropriate to analyze results.

The number of subjects in this study is not enough to produce statistically significant results. Another factor lacking in the data analysis are confidence intervals.

Review – Confidence index ranking:

Moderate to low

DRAFT

CLINICAL REVIEW FORM

Author: Linn, W.S., Shamoo, D.A., Peng, R.C., Clark, K.W., Avol, E.L., Hackney, J.D.	**Internal ID:** 315

Title:Responses to Sulfur Dioxide and Exercise by Medication-Dependent Asthmatics: Effect of Varying Medication Levels

Year:1990

Abstract: Twenty-one volunteers with moderate to severe asthma were exposed to sulfur dioxide (SO_2) at concentrations of 0 (control), 0.3, and 0.6 ppm in each of three medication states: (1) low (much of their usual asthma medication withheld), (2) normal (each subject on his own usual medication schedule), and (3) high (usual medication supplemented by inhaled metaproterenol before exposure). Theophylline, the medication usually taken by subjects, was often supplemented by beta-adrenergics. Exposures were for 10 min and were accompanied by continuous heavy exercise (ventilation approximately 50 l/min). Lung function and symptoms were measured before and after exposure. With normal medication, symptomatic bronchoconstriction occurred with exercise and was exacerbated by 0.6 ppm SO_2, as reported for mildly unmedicated asthmatics studied previously. Both baseline and post-exposure lung function were noticeably worse in the low-medication state. High medication improved baseline lung function and prevented most bronchoconstrictive effects of SO_2/exercise. High medication also increased heart rate and apparently induced tremor or nervousness in some individuals.

Objective: The purpose of this study was to extend understanding of asthmatic's responses to sulfur dioxide – specifically, the influence of common asthma medications on those responses- to provide further guidance for air quality regulatory decisions and for clinical management of asthmatics exposure to community or occupational air pollution.

Three practical questions were addressed: 1. How do moderate/severe asthmatics response when they are adhering to their usual medication regimen 2. Howe do their responses change in a "low" medication state 3. How do their responses change if they receive their usual medications plus extra prophylactic medication prior to SO_2 exposure/exercise.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0ppm (control), 0.3ppm, 0.6ppm	10 minutes	15 female 6 male	18-50 yr	21	asthmatic	n/a	n/a

Observations:

Group mean SRaw and $FEV_{1.0}$ varied significantly by exposure atmosphere, medication state and time of testing. SO_2 affected subjects' airways substantially. Function changes were largely reversed within 30 min after exposure, either spontaneously or with the assistance of bronchodilator medication.

In terms of group means, administration of metaproterenol before exposure improved lung function as compared with baseline, and completely prevented bronchoconstrictive effects of exercise in clean air or in 0.3ppm SO_2. Slight bronchoconstriction occurred during exposures to 0.6ppm SO_2, i.e., 0.6 ppm exposure partly reversed the function improvements brought about by metaproterenol preexposure; mean post-exposure lung function measurements still showed net improvements from baseline.

In the normal – medication state, exercise induced substantial bronchoconstriction in clear air, a slightly greater response when exposed to 0.3ppm SO_2 and a markedly greater response in the presence of 0.6ppm SO_2.

In the low medication state, baseline lung function was noticeably worse than in the normal medication state. Mean function changes in response to exercise and SO_2 were approximately the same with low medication as with normal medication. Therefore, because of the lower baseline, mean post-exposure lung function was markedly worse in the low medication state.

Resting heart rate at baseline did not vary significantly with SO_2 or medication conditions. Heart rates during exposure did not vary significantly with SO_2concentration.

Changes in specific symptoms listed on the questionnaire usually paralleled physiologic changes, which showed significant variation attributable to $SO_2$and medication state. In the high medication state, symptoms did not increase during exposure to clean air; symptoms increased slightly in 0.3ppm SO_2 despite the lack of physiologic response and showed slightly larger increase in 0.6 ppm SO_2. High medication also increased heart rate and apparently induced tremor or nervousness in some individuals.

In the normal medicated group, symptoms increased modestly during exposure to clean air or 0.3ppm SO_2 and increased appreciably more with 0.6ppm SO_2. A similar dose-response was observable in the low medication state, but subjects were more symptomatic both before and during exposure. Elective medication use in the first hour after exposure also increased with SO_2 concentration, and it increase in normal and low medication states as compared with high med. State.

*Statistically significant

Author's Conclusions:

"The present work, though not intended to elucidate mechanisms, provides a database to help validate current and future mechanistic hypotheses."
"Our results and related findings suggest that protection may be expected with a broad range of asthmatic patients and beta-adrenergic drugs."
"Subjects' usual medications did not prevent bronchoconstrictive response to exercise and SO_2. Theophylline does not block SO_2 /exercise induced bronchoconstriction effectively, at least not at the comparatively low plasma concentrations observed."
"Asthmatics can establish a medication schedule that provides effect protection with few side effects."
"This experiment reinforces ours and others' previous work in suggested that physiologic mechanisms of exercise-induced bronchoconstriction have much in common. Both effects are very rapid in onset, fairly quick to reverse once the stress is removed, observable in a large fraction of asthmatics, variable in intensity between individuals, readily blocked or reversed by beta-adrenergic bronchodilators, but not as readily blocked by theophyline."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(more info needed)** Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? **(-) subjects were paid** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **((no info)** Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? **(no info)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	**(-) reference to previous study** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(-) 0ppm was control** Was a control group assayed? (+) Is the control regime appropriate? **(+) double** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate?

	(+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? **(some)** Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? -) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

In this study the responses to sulfur dioxide and exercise were reported for varying medication levels. With normal medication, symptomatic bronchoconstriction occurred with exercise and was exacerbated by 0.6 ppm SO_2, as reported for mildly unmedicated asthmatics studied previously. Both baseline and post-exposure lung function were noticeably worse in the low-medication state. High medication improved baseline lung function and prevented most bronchoconstrictive effects of SO_2/exercise. High medication also increased heart rate and induced tremor or nervousness. The results are questionable because the subjects were clinically heterogeneous and the sample size was small and self-selected.

Confidence index:
The subjects were self selected, some were recruited from previous studies, the sample size was small and the subjects were paid for their participation.
The subjects were instructed to not use medication in some cases, but not all subjects reported adhering to this rule.
SO_2 was detectable by odor which may have affected symptom scoring.
Plasma was analyzed for the presence of theophylline.
This study utilized a range of exposure levels and clearly characterized subjects. It is unclear whether the sample size would be large enough to extrapolate results to a larger population. The study design characterized method of recruitment but did not clarify whether any subjects withdrew prior to experimentation. Confidence intervals are not reported nor were any standard deviations given in the data analysis. The author fails to mention whether preliminary trials were done to establish exposure rates. Also, temperature, humidity, and SO_2 concentrations were stated, but the author failed to mention airflow.

Review – Confidence index ranking:

Low to moderate

CLINICAL REVIEW FORM

Author: Linn, W.S., Avol, E.L., Shamoo, D.A., Venet, T.G., Anderson, K.R., Whynot, J.D., Hackny, J.D.	**Internal ID:** 316

Title: Asthmatics' Responses to 6hr Sulfur Dioxide Exposures on Two Successive Days

Year:1984

Abstract: Asthmatic volunteers (N = 14) aged 18 to 33 yr with documented sensitivity to sulfur dioxide (SO_2) were exposed in a chamber to 0.6 ppm SO_2 for 6-hr periods on 2 successive days. Similar exposures to purified air, 1 wk later or earlier, served as controls. Subjects exercised heavily (target ventilation rate 50 L/min) for 5 min near the beginning of exposure (early exercise) and for an additional 5 min beginning after 5-hr of exposure (late exercise). At all other times, they rested. Body plethysmographic measurements and symptom questionnaires were administered pre-exposure, after each exercise period, and hourly during rest. Bronchoconstriction and lower respiratory symptoms were observed during or immediately following exercise--to a slight extent with clean air, and to a more marked extent with SO_2. Bronchoconstriction and symptoms were modestly less severe on the second day of SO_2 exposure than on the first day, but there were no meaningful differences in response between early and late exercise periods on either day.

Objective: As a first step toward better documentation of prolonged SO_2 exposures' effects, we studied physiological and clinical responses of 14 young adult asthmatic volunteers during 6hr exposures to 0.6ppm SO_2 on 2 successive days.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0ppm and 0.6ppm	6hr periods on two successive days	12 male 2 female	18-35y	14	Asthmatic	n/a	n/a

Observations:

Substantial bronchoconstrictive responses were seen only immediately after exercise. These responses occurred in clear air as well as in SO_2, but were much greater in SO_2, producing statistically significant main effects of time and atmosphere and a significant interaction between them. There were no large differences in response between early (0hr) and late (5hr) exercise in SO_2, or between the first and second days of SO_2exposure. However, the minor variations that did occur produced a few statistical interaction of at least marginal significance.

Both SRaw and SGaw during exercise showed the expected significant differences between clean air and SO_2 exposures; neither showed significant differences between early and late exercise.

The magnitude of response to exercise in SO_2varied considerable among individuals.
The only statistically significant variation in group mean symptom scores was between clean air days and SO_2days. During exercise in SO_2 ,the typical

symptoms were sufficient to impair subjective well being to a meaningful extent, but not so severe as to prevent subject from completing the exposure protocol as planned. The mean overall score increase to the time when symptoms were worst was 20 points less on the second than on the first SO_2 day. Although this difference was not statistically significant, it suggests a modest mitigation of one or more symptoms in the typical subject on the second day. Symptom score increases during specific exercise periods showed no significant difference between late and early exercise on either SO_2epxoure day. However, both exercise periods on the second SO_2day produced 10 to 15 points less increase than occurred on the first SO_2 and this difference was statistically significant .

At the end of exposure, the mean symptom score was always minimally lower at the beginning of exposure, and the differences showed no significant variation. An hours rest typically reversed all symptoms.

Despite the absence of SO_2related symptoms immediately following exposure, there was a tendency toward less favorable clinical states in the week following exposure, relative to the clean air control studies. The mean post exposure clinical score was significantly higher after SO_2 although the difference was small. These results varied for each individual.

Bronchoconstriction and lower respiratory symptoms were observed during or immediately following exercise – to a slight extent with clean air, and to a more marked extent with SO_2.

Bronchoconstriction and symptoms were moderately less severe on the second day of SO_2 exposure than on the first day, but there were no meaningful differences in response between early and late exercise periods on either day.

Lower respiratory symptoms accounted for most of the response at the end of the 6-hr exposure period.

*Statistically significant

Author's Conclusions:

"The present physiologic results suggest that the immediate bronchoconstrictive response to exercise in SO_2 usually is not markedly affected by SO_2expsoure history during the preceding 24 hr or more. The SGaw data suggest a light mitigation of response on the second of 2 consecutive days' exposures to 0.6ppm."
"More extensive studies of prolonged SO_2 exposures might be useful for assessment of public-health risks. Such studies would be ethically and practically feasible, judging from our present results. No alarming responses were found in 12 hr cumulative exposures over 2 days, but there was at least a slight hint that effects may sometimes be greater than would be the case in single brief exposures."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (-) no info on incentive Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?

	(+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? **(not clear)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	**(-) reference to details** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined **(-) reference to details)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(no info)** Were the subjects randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(0ppm was control)** Was a control group assayed? (+) Is the control regime appropriate? (+) **double** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? **(some)** Was individual data reported for each subject? (+) **no reason given** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate?

	(-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (more info needed) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Bronchoconstriction and lower respiratory symptoms were observed during or immediately following exercise to a greater extent with SO_2 than with clean air. Bronchoconstriction and symptoms were modestly less severe on the second day of SO_2 exposure than on the first day, but there were no meaningful differences in response between early and late exercise periods on either day. The long term results were small and inconsistent enough that they might be attributable to chance, or to preferential recall of symptoms after the clinically stressful SO_2 experience.

Due to the small number of subjects utilized, results are not necessarily valid for a larger population. Future studies might consider extending investigations to a more diverse population.

Confidence index:

The study was generally well done. However, the small self-selected group of subjects makes it impossible to generalize the results. Also it can't be determined if some of the inconsistent results are real or a chance occurrence. There is no mention of whether the subjects were randomly assigned.

The literature review was fairly extensive.

There were 12 male and only 2 female in the study.

Medication use varied among the subjects.

No control group was assayed in this study. Information on how exposure levels were set is a little vague. No details on measurement equipment were given.

Review – Confidence index ranking:

Low to moderate

CLINICAL REVIEW FORM

Author: Lawther, P.J., Macfarlane, A.J., Waller, R.E., Brooks, A.G.F. **Internal ID:** 317

Title: Pulmonary Function and Sulphur Dioxide, Some Preliminary Findings

Year:1975

Abstract: In an extensive series of experiments, involving a total of twenty-five health normal subjects, increases in airways resistance have been seen when low concentrations (1-3ppm) of SO_2 have been inhaled deeply, and after higher concentrations (5-30 ppm) have been inhaled normally. Deep breathing was used to increase the inspiratory velocity to overcome absorption in the upper respiratory tract, thereby achieving better penetration of the SO_2 into the lung. A wide range of sensitivities to SO_2 was found among the subjects, and in general, the changes in airways resistance were short-lived. The relevance of these results to the effects of urban atmospheres containing SO_2 is discussed.

Objective: The present paper reports the results of seven interrelated experiments with sulphur dioxide and pulmonary function to explore the possible effects of sulphur dioxide at realistic concentrations and to investigate dose-response relationships.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Experiment 1- 1ppm	1 hour	9 male 4 female	18-47y	13	Healthy	n/a	n/a
Experiment 2- 1ppm	Not clearly stated	8 male 4 female		12			
Experiment 3- 1ppm	Varied	9 male 5 female		14			
Experiment 4 – 3ppm SO_2	Varied	12 male 5 female		17			
Experiment 5- 5-30ppm SO_2	10 min at each concentration	9 males 5 female		14			
Experiment 6- 5-30ppm and CO_2	10 minutes each	6 male 4 female		10			

Experiment 7-30ppm		6 male 4 female (same subjects as Exp. 6)					

Observations:

Experiment 1: Group Exposures in a Chamber to 1ppm SO_2 (Normal breathing)
The exposure to 1ppm SO_2 for one hour caused no significant change in any of the variables measured.
After the first hour of breathing filtered air, there was a small but significant decrease in SRaw together with a slight increase in FVC; subsequent exposure to SO_2 for one hour caused no significant change in any of the variables measured.

Experiment 2: Hyperventilation in an atmosphere of 1 ppm SO_2
For the group as a whole there as a small but significant increase in SRaw following the deep breathing with air, and a further increase after deep breaths of SO_2 at 1ppm. None of the other variables was significantly affected by the deep breaths of SO_2. The response of individual subjects varied; one had a threefold increase in SRaw. Tests were repeated excluding this one subject and there was still a significant increase in the group mean SRaw. A dose-response relationship was seen between SRaw and the number of breaths taken both for SO_2 and for air.

Experiment 3: Exposure to 1 ppm SO_2in the Plethysmograph
The results of this experiment were inconclusive. The changes in SRaw after eight deep breaths of air or 1ppm SO_2 were small and not uniform.

Experiment 4: Sequential Deep Breathing of 3 ppm SO_2
The subjects varied in their sensitivity to SO_2. The variance due to differences between subjects, between gases (air and SO_2) and between numbers of breaths were all highly significant ($p<0.001$)).

Experiment 5: Determination of Individual Responses to SO_2in Quiet Breathing
For each subject the differences between the mean of the preexposure values and the first set after exposure were highly significant for 11 of the 14 subjects. This was seen in seven subjects at 10ppm, with a t 15 ppm, nine at 20 ppm and 10 at 30ppm. The greatest increase in SRaw was seen immediately after exposure, and in general, the greater the increase, the longer it took to return to normal.

Experiment 6: Determination of Response to SO_2 with added CO_2
The results were not significant.

Experimental 7: Changes in SRaw during Exposure – 30 ppm
Four of the ten subjects showed a sharp increase in SRaw in the early part of the experiment with a return towards the control level later, even though exposure to 30 ppm SO_2 continued.

Increases in airways resistance were seen when low concentrations (1-3 ppm) of SO_2 were inhaled deeply, and after higher concentrations (5-30 ppm) were inhaled normally.

Deep breathing was used to increase the inspiratory velocity to overcome absorption in the upper respiratory tract, thereby achieving better penetration of the SO_2 into the lung.

A wide range of sensitivities to SO_2 was found among the subjects, and in general, the changes in airways resistance were short-lived.

*Statistically significant

Author's Conclusions:

"The relevance of these results to the study of the effects of air pollution on the health of the public will readily be discerned. We have reported changes in airways resistance in response to exposure to sulphur dioxide in concentrations in the range 1-3 ppm; such concentrations may be encountered in exceptional circumstances in some towns. Whilst the changes described above were of no clinical significance to the subjects, and indeed were barely perceived by some, any increase in airways resistance could, in some diseased lungs, further disturb the relationship between ventilation and perfusion with undesirable clinical consequences."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (not stated) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (-) Were the chosen exposure levels appropriate to investigate the primary objective? (not stated) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (no info) Were both nominal and actual exposure concentrations recorded? (duration varied) Was the duration of exposure appropriate to investigate the primary objective? (-) Was the duration of exposure precisely defined? (-) Was the mode of administration appropriate? (-) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (not stated) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?

C. Equipment:	**(some)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined **(-)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(-)** Were the subjects randomly assigned to exposure groups? **(-)** Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) **(-)** Was a control group assayed? **(-)** Is the control regime appropriate? **(varied)**Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) **(-)** Was the length of follow-up appropriate? **(-)** Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	**(+)** Were appropriate endpoints examined? **(-)** Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(+)** Were accepted assessment methods employed? **(-)** Were assessment measures sufficiently sensitive? **(-)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? **(-)** Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(some)** Was individual data reported for each subject? **(-)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	**(more info needed)** Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(more info needed)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	**(objective not clear)** Was the original objective addressed? **(-)** Are the results novel? **(-)** Was the design of the study appropriate to investigate the primary objective? **(-)** Were assumptions reasonable? **(-)** Are the methods reproducible?

	(-) Did either the study design or the statistical analysis limit confounding variables? (-) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (yes) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study was actually a series of experiments performed one after another. Consequently, results are difficult to interpret. This was amplified by the variations in subject response and physiological make-up including airway resistance.

The findings were very inconsistent and inconclusive for all 7 experiments. None of the findings were clinically significant. See below for study design issues.

Confidence index:

There is extremely limited information provided for each experiment. The methods and results are described together.

Some of the subjects were smokers.

The subjects were not chosen or assigned randomly.

The methods of exposure varied for each experiment and for some experiments was not stated.

The duration of exposure is not always clear.

In some studies, SO_2 was breathed in through a bag and the effects were calculated based on the number of breaths taken by each subject before an effect was observed.

The variability between individuals was significant for all experiments.

There were some extended delays between exposure and measurements.

Not all of the experiments were blinded.

Some experiments used noseclips, some used mouthpieces, some used bags, and it was different for each experiment.

The SO_2 levels were very high.

The subjects were members of the unit staff doing the research.

Only three of the total 25 subjects were available for the whole series.

The experiment is its entirety would be difficult to reproduce.

No information was given on the equipment used. Data collection and assessment was not thorough.

Review – Confidence index ranking:

Low

CLINICAL REVIEW FORM

Author: Islam, M.S., Oberbarnscheidt, J., Schlipkoter, H.W. | **Internal ID:** 318

Title:Non-specific Airway Responsiveness to Hyperventilation of Low Doses of Sulfur Dioxide and Cold Air of Non-smoking Healthy Volunteers of Different Ages

Year:1994

Abstract: We examined the effect of hyperventilation of sulfur dioxide air and cold air for 5 minutes on airway responsiveness of 37 (12 females and 25 males) healthy nonsmoking volunteers of different ages. Body plethysmographic measurements of airway resistance and intrathoracic gas volume were performed before, 3, 10 and 20 minutes after the end of HV of SO_2-air or cold air. Specific airway resistance, product of Raw x ITGV, was used to evaluate the airway responsiveness. The mean (standard error) of percent changes of SRaw were 121, 45 and 25 at 3, 10 and 20 minutes after the end of HV of SO_2-air. The corresponding values were 52(8), 25(5) and 19(3) after HV of cold air. The differences in change in Raw between HV of SO_2 and cold air were statistically significant for the 3 and 10 minute values. Hyperventilation of SO_2 and cold air produced changes in Raw above 100% between the HV of SO_2 and cold air was statistically significant. We observed a decreasing order of airway responsiveness with increasing age following HV of SO_2 but not after cold air. We conclude that airway responsiveness to SO_2 is poorly related to the response to cold air.

Objective: We undertook this study to examine the influence of HV of low doses of $SO_2$and cold air on airway responsiveness of non-smoking healthy volunteers of different ages.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean SO_2: $1.91 \pm 0.14 mg/m^3$	5 min	12 females 25 males	Females: 25.6 ± 10.3y Males 27.7 ± 11.7y	37	Nonsmoking and healthy	No observed adverse effect level	Lowest observed adverse effect level

Observations:

Changes of specific airway resistance after hyperventilation of $SO_2$and cold air

The differences between SO_2-air and cold air responses were statistically significant after 3 ($p<0.01$) and 10 ($p<0.05$) mins of HV, but it was not significant after 20 minutes. Full recovery was reached in 14 (37.8%) volunteers within 20 mins after HV of SO_2-air mixture. Other volunteers needed up to 40 mins for full recovery.

Frequency of changes of specific airway resistance greater than 100%

We observed changes in SRaw above 100% in 14 (37.8%) following HV of SO_2 air mixture. The difference in frequencies of responses greater than 100% between HV of SO_2-air and cold air, was statistically significant ($p<0.05$).

Relative Changes in different age groups

There was a decreasing order of airways responsiveness due to HV of SO_2-air with increasing age, but this was not found in the case of HV of cold air. However, the age related gradient of SO_2 responses were not statistically significant.

*Statistically significant

Author's Conclusions:

"We conclude that airway responsiveness to HV of SO_2 differs significantly from the responses induced by HV of cold air, both in terms of frequency and magnitude of changes in SRaw. So, the term "non-specific" airway responsiveness can poorly define airway responses induced by HV of SO_2 and cold air. Our data demonstrate that HV of cold air as a provocation test may fail to detect atmospheric pollutant-related airway hyper responsiveness. Our results suggest that the airway responsiveness to SO_2 is significantly ($p<0.05$) more frequent among volunteers below than above 30 years of age, but this is not the case in HV of cold air."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	. (more info needed) Is the number of subjects sufficient to produce statistically significant results\\ . (+) No work history Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? . (-) Are the subjects a representative sample of the population to which extrapolations will be made? . (-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? . (no info) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	. (-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? . (-) Was there a gradient of exposure levels? . (+) Were the chosen exposure levels appropriate to investigate the primary objective? . (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? . (+) Were both nominal and actual exposure concentrations recorded? . (+) Was the duration of exposure appropriate to investigate the primary objective? . (-) Was the duration of exposure precisely defined? . (+) Was the mode of administration appropriate? . (+) Was the frequency of administration appropriate to investigate the primary objective? . (+) Was evidence provided to justify the dosing regime? . (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?

C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(no info)** Were the subjects randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(cold air was control)** Was a control group assayed? (+) Is the control regime appropriate? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable? **(more detail needed)** Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables?

	. (+) Do the conclusions make biological sense? . (-) Are the extrapolations reasonable? . (+) Is the paper in a peer-reviewed journal? . (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The objective of the study was to examine the airway responsiveness of healthy persons to HV of SO_2 – air and cold air. The results show significantly greater increases in SRaw following HV of SO_2 –air than of cold air. The results also revealed an age-dependent airway responsiveness to SO_2. There is some question with the selection of the subjects preventing these results from being generalizable to other groups.

Results are in good agreement with the findings in asthmatic patients in that there are high variability and a poor relationship between airway response to SO_2 and MCh. Data suggest that HV of cold air as a provocation test may fail to detect atmospheric pollutant-related airway hypersensitivity.

The difference in frequencies of ΔSRaw between HV of SO2 and HV of cold air indicate that airway responsiveness to SO_2 is poorly related to the response to cold air. As well, results show age dependent airway responsiveness to SO_2; this result was not paralleled when HV of cold air was studied. Additional studies with a broader population sample can be done to expand on this conclusion.

Confidence index:

The study was well designed. However, the method section could have used more detail. Detail is included in a previous study referenced by the authors.

The experimental design was well described with diagrams.

Mouthpieces and nose clips were used, so the breathing wasn't necessarily natural.

The $SO_2$was combined with cold air.

It is not mentioned if the subjects were randomly assigned.

The subjects were healthy university and high school students and members of the institute conducting the investigation. There are no other details on how the subjects were chosen.

This study limits confounding factors such as gender age, and pre-trial health. Statistical analysis was effective in producing significant results.

Review – Confidence index ranking:

Moderate to low

CLINICAL REVIEW FORM

Author: Islam, M.S., Oberbarnscheidt, J., Schlipkoter, H.W.	**Internal ID:** 318
Title:Non-specific Airway Responsiveness to Hyperventilation of Low Doses of Sulfur Dioxide and Cold Air of Non-smoking Healthy Volunteers of Different Ages	
Year:1994	
Abstract: We examined the effect of hyperventilation of sulfur dioxide air and cold air for 5 minutes on airway responsiveness of 37 (12 females and 25 males) healthy nonsmoking volunteers of different ages. Body plethysmographic measurements of airway resistance and intrathoracic gas volume were performed before, 3, 10 and 20 minutes after the end of HV of SO_2-air or cold air. Specific airway resistance, product of Raw x ITGV, was used to evaluate the airway responsiveness. The mean (standard error) of percent changes of SRaw were 121, 45 and 25 at 3, 10 and 20 minutes after the end of HV of SO_2-air. The corresponding values were 52(8), 25(5) and 19(3) after HV of cold air. The differences in change in Raw between HV of SO_2 and cold air were statistically significant for the 3 and 10 minute values. Hyperventilation of SO_2 and cold air produced changes in Raw above 100% between the HV of SO_2 and cold air was statistically significant. We observed a decreasing order of airway responsiveness with increasing age following HV of SO_2 but not after cold air. We conclude that airway responsiveness to SO_2 is poorly related to the response to cold air.	
Objective: We undertook this study to examine the influence of HV of low doses of $SO_2$and cold air on airway responsiveness of non-smoking healthy volunteers of different ages.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean SO_2: $1.91 \pm 0.14 mg/m^3$	5 min	12 females 25 males	Females: 25.6 ± 10.3y Males 27.7 ± 11.7y	37	Nonsmoking and healthy	No observed adverse effect level	Lowest observed adverse effect level

Observations:

Changes of specific airway resistance after hyperventilation of $SO_2$and cold air

The differences between SO_2-air and cold air responses were statistically significant after 3 ($p<0.01$) and 10 ($p<0.05$) mins of HV, but it was not significant after 20 minutes. Full recovery was reached in 14 (37.8%) volunteers within 20 mins after HV of SO_2-air mixture. Other volunteers needed up to 40 mins for full recovery.

Frequency of changes of specific airway resistance greater than 100%

We observed changes in SRaw above 100% in 14 (37.8%) following HV of SO_2 air mixture. The difference in frequencies of responses greater than 100% between HV of SO_2-air and cold air, was statistically significant ($p<0.05$).

Relative Changes in different age groups

There was a decreasing order of airways responsiveness due to HV of SO_2-air with increasing age, but this was not found in the case of HV of cold air. However, the age related gradient of SO_2 responses were not statistically significant.

*Statistically significant

Author's Conclusions:

"We conclude that airway responsiveness to HV of SO_2 differs significantly from the responses induced by HV of cold air, both in terms of frequency and magnitude of changes in SRaw. So, the term "non-specific" airway responsiveness can poorly define airway responses induced by HV of SO_2 and cold air. Our data demonstrate that HV of cold air as a provocation test may fail to detect atmospheric pollutant-related airway hyper responsiveness. Our results suggest that the airway responsiveness to SO_2 is significantly ($p<0.05$) more frequent among volunteers below than above 30 years of age, but this is not the case in HV of cold air."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results\ (+) No work history Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (no info) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (-) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?

C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(no info)** Were the subjects randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) **(cold air was control)** Was a control group assayed? (+) Is the control regime appropriate? (-) Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable? **(more detail needed)** Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables?

	. (+) Do the conclusions make biological sense? . (-) Are the extrapolations reasonable? . (+) Is the paper in a peer-reviewed journal? . (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The objective of the study was to examine the airway responsiveness of healthy persons to HV of SO_2 – air and cold air. The results show significantly greater increases in SRaw following HV of SO_2 –air than of cold air. The results also revealed an age-dependent airway responsiveness to SO_2. There is some question with the selection of the subjects preventing these results from being generalizable to other groups.

Results are in good agreement with the findings in asthmatic patients in that there are high variability and a poor relationship between airway response to SO_2 and MCh. Data suggest that HV of cold air as a provocation test may fail to detect atmospheric pollutant-related airway hypersensitivity.

The difference in frequencies of ΔSRaw between HV of SO2 and HV of cold air indicate that airway responsiveness to SO_2 is poorly related to the response to cold air. As well, results show age dependent airway responsiveness to SO_2; this result was not paralleled when HV of cold air was studied. Additional studies with a broader population sample can be done to expand on this conclusion.

Confidence index:

The study was well designed. However, the method section could have used more detail. Detail is included in a previous study referenced by the authors.

The experimental design was well described with diagrams.

Mouthpieces and nose clips were used, so the breathing wasn't necessarily natural.

The $SO_2$was combined with cold air.

It is not mentioned if the subjects were randomly assigned.

The subjects were healthy university and high school students and members of the institute conducting the investigation. There are no other details on how the subjects were chosen.

This study limits confounding factors such as gender age, and pre-trial health. Statistical analysis was effective in producing significant results.

Review – Confidence index ranking:

Moderate to low

NON-CLINICAL REVIEW FORM

Author: Knorst, M.M., Kienast, K., Grob, S., Fries, B., Muller-uernheim J., Ferlinz, R.	**Internal ID:** 319

Title: Chemotactic response of human alveolar macrophages and blood monocytes elicited by exposure to sulfur dioxide.

Year:1996

Abstract: An experimental study was undertaken to investigate the in vitro effect of sulfur dioxide on the chemotactic activity of alveolar macrophages (AM) and blood monocytes (BM). The cells were placed on a polycarbonate membrane and exposed to SO_2 0.5, 1.5 and 2.5 ppm for 15 min. Control experiments were performed with exposure of the cells to synthetic air with 5% CO_2. After gas exposure the cells were incubated with the chemotactic active agent C5a in 5% carbon dioxide (CO_2) at 37 degrees C for 60 min. The numbers of AM and BM passing actively through the membrane were quantified using light microscopy. Our results show a dose-dependent reduction in the migration rate of cells under SO_2 exposure. SO_2 0.5 ppm induced a 29% and SO_2 2.5 ppm a 53% decrease in migration of AM compared with the control exposure to synthetic air ($P < 0.01$). Identical experiments with BM resulted in a decrease in migration of up to 57% ($P < 0.01$). At SO_2 concentrations of up to 2.5 ppm no significant cytotoxic effects were observed for AM or BM. The data demonstrate that exposure to SO_2 may reduce the chemotactic activity of AM and BM. Our results further suggest that the decrease in cell migration induced by SO_2 is due to changes in chemotactic mechanisms and not to cell death.

Objective: It was the purpose of our in vitro experiments to study the effect of SO_2 on the chemotactic activity of AM and blood monocytes (BM)

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0.5, 1.5, 2.5ppm	15 min for each level	human		59±10 years	Macrophages: 1 woman 4 men	Macrophages: 5	Bronchial carcinoma	No observed adverse effect level	Lowest observed adverse effect level
				32±10 years	Mononuclear cells: 2 women 5 men	Mononuclear cells: 7	Not stated		

Observations:

Chemotactic Activity of AM

Exposure to SO_2 of up to 2.5ppm induced a significant dose-dependent decrease in AM migration ($p<.01$). Exposure to 0.5ppm caused a 29% decrease in cell migration compared with the values of the control experiment using synthetic air. After exposure to SO_2 1.5ppm and 2.5ppm a 44% and 53% decrease respectively was recorded in the chemotactic activity of AM.

Chemotactic activity of BM

Exposure to SO_2 0.5ppm caused a 32% decrease in the chemotactic activity of BM while after exposure to SO_2 1.5ppm only 54% of the control cell migration was recorded. The maximum decrease of 57% was induced by exposure of the cells to SO_2 2.5ppm ($p<0.01$).

Cell viability

No alteration in cell viability was observed after exposure of AM to synthetic air and SO_2 0.5 or 1.5ppm. After exposure to SO_2 2.5ppm a small and statistically insignificant cell toxicity was noted ($p>.05$).

*Statistically significant

Author's Conclusions:

"In the present study, we have demonstrated that short-term in vitro exposure to SO_2 produces changes in AM and BM chemotactic activity. 15 minute exposure to SO_2 at a range from 0.5 to 2.5 ppm resulted in a dose-dependent decrease in AM and BM migration of up to 53% and 57%, respectively. The decrease in cell migration was already significant after exposure to SO2 0.5ppm for both cell types…the viability of the cells was not significantly altered after exposure to SO_2. These results suggest that the observed changes in cell migration are more probably related to functional damage of the cells induced by SO_2 than to a loss in cell viability."

"In conclusion, the reported data demonstrate that short-term in vitro exposure to SO_2 reduces the chemotactic performance of AM and BM without altering cell viability. As to whether the observed decrease in chemotactic activity of the phagocytic cells after SO_2 exposure also plays a role in *in vivo* situations can not be demonstrated on the basis of the results obtained by the present in vitro study."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	(more info needed) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+) b) Age *Guideline: Adult, i.e..8-12 weeks.*

	(+) c) Sex (+) d) Pre-test health ↳ *Guideline: Females should be nulliparous and non-pregnant.* **(no info)** e) Weight variation ↳ *Guideline: Below 20% variation* **(no info)** f) Source ↳ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? ↳ *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(not stated)** Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? **(n/a)** If exposure chambers were employed, were animals exposed during the equilibration period? ↳ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? **(not stated)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(n/a)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ↳ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(n/a)** Was the number of animals grouped in a single chamber stated?

	Guideline: Groupings should permit clear observation of each animal. **(n/a)** Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* **(n/a)** Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* **(n/a)** Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(+)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(-)** Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(n/a)** Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* **(+)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(n/a)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(n/a)** Was a control group assayed? **(+)** Is the control regime appropriate? **(n/a)** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	**(+)** Were appropriate endpoints examined? **(+)** Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(+)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a)** Was the nature and severity of symptoms graded against an explicitly

	defined scale? **(not stated)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? **(n/a)** Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? **(more details needed)** Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? **(more detail needed)** Do the conclusions make biological sense? **(no extraps made)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study presented evidence that short term in vitro exposure to SO_2 produces changes in AM and BM chemotactic activity. The exposure reduced the chemotactic performance of AM and BM without altering cell viability. These results may provide further evidence as to the mechanism of SO_2 exposure, but it cannot be assumed that in vivo studies would produce the same results and further studies are needed to verify the results.

Confidence Index:

Direct exposure of the cells to gas was permitted.

There was no info on the monitoring of SO_2 during the exposure.

The subjects chosen for the isolation of human alveolar macrophages were significantly older than the subjects that donated the blood mononuclear cells.

The subjects that donated the macrophages had bronchial carcinoma. These results may differ in macrophages from healthy subjects.

It was not clear how subjects were selected or if they were informed of experimental risks.
As this is an in vitro study, a lot of the Housing/Feeding requirements did not need to be met. No evidence was provided to justify the dosing regime and no raw data or individual data was reported.

Review – Confidence index ranking:

Low

CLINICAL REVIEW FORM – HUMAN Experimental

Author: Kienast, K., Riechelmann, H., Knorst, M., Schlegel, J., Muller-Quernheim, J., Scellenberg, J., Reflinz, R.	**Internal ID:** 320

Title:An experimental model for the exposure of human ciliated cells to sulfur dioxide at different concentrations

Year:1994

Abstract: Mucociliary transport is an important nonimmunological defense mechanism of the respiratory tract. The aim of this study was to investigate the effect of sulfur dioxide (SO_2) at different concentrations on ciliary beat frequency (CBF). Ciliated cells were obtained from 12 volunteers by nose brush. CBF was quantified using video-interference microscopy. The cells were placed on a polycarbonate membrane in contact with the surface of a reservoir filled with RPMI 1640 (bicarbonate buffered) or Ringer's (electrolyte) solution, allowing the cells to be supplied by capillarity. In an exposure chamber the cells were exposed for 30 min to SO_2 2.5-12.5 ppm at 37 degrees C and 100% air humidity. SO_2 induced a dose-dependent decrease in CBF of the cells cultured in Ringer's solution. SO_2 at 2.5 ppm caused a 42.8% decrease and at 12.5 ppm a 96.5% decrease (8.1 +/- 0.24 versus 0.28 +/- 0.20 Hz). CBF of cells cultured in RPMI 1640 was reduced only moderately after 12.5 ppm SO_2 exposure (7.9 +/- 0.26 versus 6.70 +/- 0.30 Hz). In Ringer's solution a decrease in pH was observed after 30 min of SO_2 exposure to 12.5 ppm to a minimum value of 3.6. By contrast, the pH of RPMI 1640 remained constant at 7.5 under identical conditions. After adding RPMI 1640 to Ringer's solution, CBF increased in parallel to the pH to control values (5.0 ppm: 4.64 +/- 0.45 to 8.51 +/- 0.60 Hz). These data suggest that the highly water-soluble SO_2 reversibly eliminates CBF in correlation with a decrease in pH.

Objective: We developed an experimental model using a cell culture technique in gas phase with the aim of establishing the value of this method with regard to ciliary's cell response to SO_2.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
2.5-12.5ppm	30 min	7 males 5 female	32±5 yrs	Ciliated cells from 12 subjects	health		

Observations:

The ciliated cells of 12 probands were exposed to SO_2 concentrations of 2.5-12.5ppm for 30 min.
Using RPMI as the cell medium, CBF was decreased at 2.5ppm SO_2 from initially 7.9±0.26 (control exposure) to 7.3±0.24Hz; at 12.5ppm SO_2 CBF was 6.7±0.3Hz ($p<0.001$). Using Ringer's solution as culture medium, a dose-dependent decrease in CBF after an SO_2 exposure of the ciliated cells was observed. A 30min exposure to 2.5ppm SO_2 yielded a 42.8% decrease in CBF, at 12.5ppm SO_2 we recorded a 96.5% decrease in CBF at 0.28±0.20 Hz ($p<0.0005$). After exposure to 12.5ppm SO_2 there was thus no coordination of ciliary beating.

A comparison of the pH and CBF after SO_2exposure with different demonstrated a high correlation between SO_2 induced pH reduction and CBF decrease ($P<0.0005$).

CBF of cells cultured in RPM 1640 was reduced only moderately after 12.5 ppm SO_2 exposure. In Ringer's solution a decrease in pH was observed after 30 minutes of SO_2 exposure to 12.5 ppm to a minimum value of 3.6. By contrast, the pH of RPMI 1640 remained constant at 7.5 under identical conditions. After adding RPMI 1640 to Ringer's solution, CBF increased in parallel to the pH to control values. These data suggest that the highly water-soluble SO2 reversibly eliminates CBF in correlation with a decrease in pH.

*Statistically significant

Author's Conclusions:

"In summary, we used a method of cell culture in aerobiosis, which allows the target cells to be in direct contact with the atmosphere, to evaluate the biological effects of gas pollutants on ciliated cells. Our data suggest that the well-soluble SO_2 eliminates CBF in vitro as a result of increased acidification of the tissue culture. The buffer capacity of biological fluids, however, dampens this mechanism in vivo. Therefore the system introduced is suited for the in vitro investigation of the pathophysiology of cilia. The addition of body fluids to the system might expand its applicability for physiologica reactions. Our data show that cell culture in aerobiosis offers new alternatives for research on the biological effects of gas pollutants."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]
****This was an in vitro study, so the guidelines below may not all be applicable****

A. Subjects:	(+)_ Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(no info)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? **(no info)** Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(+) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(not stated)** Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime?

	(+)**for temperature** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(n/a)** Were the subjects randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(n/a)** Was a control group assayed? (+) Is the control regime appropriate? **(n/a)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(n/a)** Was the length of follow-up appropriate? **(n/a)** Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(n/a)** Was individual data reported for each subject? **(n/a)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable?

	(-) Are the methods reproducible? **(no info)** Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? **(no extrap. Made)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The authors used a method of cell culture in aerobiosis which allowed the target cells to be in direct contact with the atmosphere, to evaluate the biological effects of gas pollutants on ciliated cells. The effects of SO_2 on ciliary beat frequency were affected by the pH which varied depending on the culture medium used. The data suggested that SO_2 eliminates CFB in vitro as a result of increased acidification of the tissue culture. The authors make no extrapolations to in vivo studies, because the buffer capacity of biological fluids will dampen this mechanism.

This study was particularly suited to determining the biological effects on target cells of toxic gases and different concentrations. This study was novel in that it used a method of cell culture in aerobiosis, allowing target cells to be directly exposed to the environment.

Confidence index:

The study is fairly well designed. However, there could be more detail in the methods section. The authors are not claiming that the results can be extrapolated to humans (in vivo exposure) They are just providing new alternatives for research on the biological effects of gas pollutants.

The method allows the cells to be exposed directly.

There is limited info on the monitoring of the SO_2 during the experiment

A full description of the reagents was not given. However, the Friedman Test was correctly used to analyze data.

The ciliated cells and airway mucus was suspended in Ringer's solution for 30 min, adjusted to pH 7.4 and 37C. Confidence intervals were not provided. Raw data were not provided either.

Review – Confidence index ranking:

Moderate to low

CLINICAL REVIEW FORM

Author: Lazarus, S.C., Wong, H.H., Watts, M.J., Boushey, H.A., Lavins, B.J., Minkwitz, M.C.

Internal ID: 321

Title: The Leukotriene Receptor Antagonist Zafirlukast Inhibits Sulfur Dioxide – induced Bronchoconstriction in patients with asthma

Year: 1997

Abstract: Inhalation of sulfur dioxide (SO_2) causes bronchoconstriction in most people with asthma. To examine the role of leukotrienes in this response, the antagonism of SO_2-induced bronchoconstriction by a single oral dose of the leukotriene receptor antagonist zafirlukast was assessed in a double-blind, placebo-controlled, two-period crossover trial in 12 subjects with mild-to-moderate asthma. Subjects had bronchial hyperresponsiveness, an FEV1 < or = 70% of predicted, and a positive response to inhaled SO_2 (an 8-unit increase in specific airway resistance on inhaling an SO_2 concentration of < or = 4 ppm (PC8SRaw). Subjects were treated with zafirlukast (20 mg) or placebo on two treatment days 5 to 14 d apart. Two and 10 hours after treatment, subjects inhaled SO_2 (0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 ppm) during eucapnic hyperventilation at 20 L/min. PC8SRaw was determined after each challenge. Blood samples were collected to assess zafirlukast plasma concentrations versus effect. PC8SRaw was significantly higher 2 h after zafirlukast compared with placebo (3.1 versus 1.5 ppm; $p = 0.02$) and remained higher 10 h after treatment with zafirlukast (2.7 versus 1.9 ppm; $p = 0.09$). An association was found between zafirlukast plasma concentrations and increases in PC8SRaw 10 h after treatment ($p = 0.001$). The safety profile of zafirlukast was not clinically different from placebo. A single 20-mg dose of zafirlukast attenuated SO_2-induced bronchoconstriction. We conclude that SO_2-induced bronchoconstriction involves release of leukotrienes and that treatment with zafirlukast attenuates the bronchoconstrictor response.

Objective: We hypothesize that cysteinyl leukotrienes contribute to the bronchoconstriction seen in SO_2 sensitive patients during exposure to SO_2 ; therefore, we conducted this trial to determine whether zafirlukast inhibits SO_2 induced bronchoconstriction in subjects with asthma.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.25, 0.5, 1.0, 2.0, 4.0, and 8.0ppm	4 minutes at each concentration Exposure occurred 2h and 10h after treatment or placebo	7 men 5 women	25-43y	12	asthmatic	No observed adverse effect level	Lowest observed adverse effect level

Observations:

Two hours after treatment with zafirlukast, the SO_2 concentration response curves were shifted to the right in 10 of 12 subjects, showing an increase in the threshold dose but no change in maximum response.

Ten hours after treatment, curves were shifted to the right in 10 of 12 subjects. Mean PC_8SRaw for all subjects was significantly higher 2h after treatment with zafirlukast compared with placebo (3.1 versus 1.5 ppm; p=0.02). The mean PC_8 SRaw remained higher 10 h after treatment, although the difference between treatments was no longer significant (2.7 versus 1.9ppm; p=0.09)

All subjects who received zafirlukast had quantifiable plasma concentrations. An association was found between zafirlukast plasma concentrations and increases in PC8SRaw 10 h after treatment (p = 0.001).

*Statistically significant

Author's Conclusions:

In summary, we have shown that a single oral dose of the leukotriene receptor antagonist zafirlukast inhibits SO_2-induced bronchoconstriction in subjects with mold-to-moderate asthma. Therefore, we conclude that SO_2induced bronchoconstriction involves leukotriene release.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(more info needed)** Is the number of subjects sufficient to produce statistically significant results? (+) **no work history** Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? **(no info)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(+) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(not clear)** Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate?

	(+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(reference to details in another paper) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (-) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (placebo) Was a control group assayed? (+) Is the control regime appropriate? (+) double Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (some) Was raw data provided to permit the reader to arrive at his/her own conclusions? (some) Was individual data reported for each subject? (n/a no withdrawals) exclusion criteria given) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed?

	(-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) **more detail needed** Are the methods reproducible? (some) , **more info needed** Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:
The findings in this study provide evidence as to the mechanism of SO_2 induced bronchoconstriction. That is, that the release of leukotrienes contributes to the response to SO_2.

Confidence index:
The sample size was relatively small.
There is no information as to where the subjects were chosen from.
There was good detail on the SO_2challenge.
The responses were measured 2hr and 10hrs after treatment. Bronchial reactivity may differ depending on the time of day.
This study effectively limits confounding factors such as pre-trial health, gender and age. Subjects were advised of experimental risks and the protocol was approved prior to the experiment. Exclusion criteria were listed and double-blinding was employed. Subjects were randomly assigned to groups.
Results were novel showing that the LTRA zafirlukast inhibits SO_2 induced bronchoconstriction and the original objective was addressed. However, confidence intervals were not reported and the sample size was not large enough to produce statistically significant trends. Individual data was reported for each subject, but no raw data was reported. Also, there was no evidence to justify the dosing regime.

Review – Confidence index ranking:

Moderate

CLINICAL REVIEW FORM

Author: Nowak, D., Jorres, R., Berger, J., Claussen, M., Magnussen, H. **Internal ID:** 322

Title:Airway Responsiveness to Sulfur Dioxide in an Adult Population Sample

Year:1997

Abstract: We determined the prevalence of airway hyperresponsiveness to sulfur dioxide (SO_2) in an adult population sample of 790 subjects 20 to 44 yr of age. Subjects were drawn randomly from the population of Hamburg, Northern Germany, within the framework of the European Community Respiratory Health Survey. In addition, we analyzed the relationship between SO_2 responsiveness and a number of risk factors, such as a history of respiratory symptoms, methacholine responsiveness, and atopy derived from skin-prick test results. SO_2 inhalation challenges were performed during isocapnic hyperventilation at constant rate (40 L x min(-1), for 3 min) with doubling concentrations of SO_2 up to a maximum concentration of 2.0 ppm. If subjects achieved a 20% decrease in FEV1 from baseline during the challenge, they were considered to be hyperresponsive to SO_2. The raw prevalence of SO_2 hyperresponsiveness within the population sample studied was 3.4% (95% confidence interval [CI]: 2.3 to 5.0%). Adjustment for nonparticipation led to an estimated prevalence of SO_2 hyperresponsiveness of 5.4%. Among subjects with hyperresponsiveness to methacholine, 22.4% (95% CI: 20.1 to 25.3) demonstrated hyperresponsiveness to SO_2. There was no significant correlation between the degrees of hyperresponsiveness to methacholine and SO_2. Predictors of a positive SO_2 response were hyperresponsiveness to methacholine ($p < 0.0001$), a positive history of respiratory symptoms ($p < 0.05$), and a positive skin-prick test to at least one common allergen ($p < 0.05$). We conclude from these data that airway hyperresponsiveness to SO_2 can be found in about 20 to 25% of subjects within the 20- to 44-yr age range who are hyperresponsive to methacholine.

Objective: It was the aim of our study to determine the prevalence of hyperresponsiveness to methacholine, as well as within the general population. In addition, we studied potential risk factors for SO_2hyperresponsiveness.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0, 0.25, 0.5, 1.0, and 2.0ppm SO_2	3 minutes each	52.7%male 48.1%female	20-44y	790	varied	varied	

Observations:

Prevalence of Airway Hyperresponsiveness to Methacholine
Valid results of methacholine challenges were obtained for 1002 subjects. The total frequency of nonspecific airway hyperresponsvieness was 22.6% (228/1008), yielding a 95% CI of 20.1-25.3%.

Prevalence of Hyperresponsiveness to SO_2

Among men, 2.5% (11 of 439) and among women, 4.6% (16 of 347) showed hyperresponsiveness to SO_2. The total prevalence was 3.4% (27 of 786) with a 95% CI of 2.3 to 5.0%. None of the subjects responded to a SO_2 concentration below 0.25ppm whereas two (females) responded to concentrations below 0.5ppm, 13 (five males and eight females) responded to concentrations below 1.0 ppm and 12 (six males and six females) responded to concentrations below 2.0ppm. Adjustment for nonparticipation led to an estimated prevalence of SO2 hyper responsiveness of 5.4%.

Relationship between Hyperresponsiveness to Methacholine and SO_2.

Among the 679 subjects who were normoreactive to methacholine, only three demonstrated more than a 20% decease in FEV_1 during the SO_2 challenge. The specificity of a negative methacholine test for predicting a negative SO_2 challenge was 99.6%. Among the 107 subjects with airway hyperresponsiveness to methacholine, 24 subjects showed hyper responsive ness to SO_2. Therefore, the sensitivity of a positive methacholine test for predicting a positive SO_2 test was 22.4%. Despite this association between methacholine and SO_2 responsiveness, the individual values of $PD_{20}FEV_1$ for methacholine and $PC_{20}FEV_1$ for SO_2 did not correlate significantly with each other .

Predictors of a positive SO_2 response were hyperresponsiveness to methacholine ($p < 0.0001$), a positive history of respiratory symptoms ($p < 0.05$), and a positive skin-prick test to at least one common allergen ($p < 0.05$).

*Statistically significant

Author's Conclusions:

Methacholine responsiveness was a predictor of hyperresponsiveness to SO_2, with low sensitivity and high specificity. In summary, we estimated the distribution of increase airway responsiveness to SO_2 in young adults. SO_2 hyperresponsiveness was found in 22.4% of subjects with airway hyperresponsiveness to methacholine. Methacholine hyperresponsiveness, respiratory symptoms, and atopy were significant predictors of a positive lung function response to SO_2.
"We conclude from these data that airway hyperresponsiveness to SO_2 can be found in about 20 to 25% of subjects within the 20 to 44 year age range who are hyperresponsive to methacholine."
"The lack of a significant correlation between the degree of airway hyperresponsiveness to SO_2 and the degree of airway hyperresponsiveness to methacholine, as found in the present study is in accord with previously published data. Similar findings have been obtained for histamine."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (+) financial compensation Was the method of recruitment appropriate? Would the participation incentive employed cause the selection

	of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(+) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+/-) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (+)Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (+) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(not stated)** Were the subjects randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) **(-) control was filtered air** Was a control group assayed? (+) Is the control regime appropriate? (-) Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams?

	(+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+) **and exclusion criteria** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? **(more detail needed)** Was the design of the study appropriate to investigate the primary objective? **(more detail needed)** Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study showed that airway hyperresponsiveness to SO_2 can be found in about 20 to 25% of subjects within the 20 to 44 yr age range who are hyperresponsive to methacholine. The prediction had low sensitivity and high specificity. Therefore, the degree of hyperresponsiveness to methacholine does not necessarily predict the airway hyperresponsiveness to SO_2. There were some issues with the study design that may affect the validity of the results.

This study faced a potential selection bias by inviting subjects to participate in clinical examinations. Results from this study indicate that subjects who participate tended to report more respiratory symptoms than those who did not.

No correlation was found between airway hyperresponsiveness to SO_2 and the degree of airway hyperresponsiveness to methacholine. The relationship between respiratory symptoms and hyperresponsiveness and the chronic morbidity of subjects with preexisting airway diseases or the long term development of chronic obstructive airway disease in subjects chronically exposed to SO_2 remains to be established.

Confidence index:

The method of randomization is not given.

The original sample of 4500 only represents 0.6% of the total population.

Subjects received financial compensation to take part in the second stage of the study.

Some of the subjects were smokers, but it is not stated how many. Subjects were only instructed not to smoke 1 hour before testing.

Nose clips were used.
There is no indication that the exposures were randomly assigned.
The authors did an analysis on other risk factors for hyperresponsiveness to SO_2.
The authors attempted to correct for selection bias that may have occurred during subject recruitment; however, these methods are not justified or validated.
Justification was provided for the concentrations of SO_2used.
Reported some consistency with other studies.
Given the large sample size, the study was effective in recording subjects characteristics such as age, health and gender. However, it was virtually impossible to ensure that subjects shared similar health characteristics, incorporating a major confounding factor into the study. In addition, no control group was clearly indicated.

Review – Confidence index ranking:

Low to moderate

DRAFT

CLINICAL REVIEW FORM

Author: Frank, N.R., Amdur, M.O., Worcester, J., Whittenberger, J.L.	**Internal ID:** 323

Title:Effects of acute controlled exposure to SO_2 on respiratory mechanics in healthy male adults

Year:1961

Abstract: Eleven healthy, adult, male volunteers were exposed on separate occasions to average levels of sulfur dioxide of 1, 5, and 13 ppm. The subjects were seated in a body plethysmograph, breathing spontaneously by mouth while measurements of respiratory mechanics were made with a esophageal catheter. Exposures lasted 10-30 minutes and, for each subject, were spaced at lest 1 month apart. With on e exception the group showed no significant increase in pulmonary flow resistance at the lowest concentration of gas. At both 5 and 13 ppm, flow resistance was elevated, the change being greater at 13 ppm. The change occurred within 1 minute of exposure, increased after 5 min but, on the average, shoed no further change after 10 min. Four subjects were exposed to 5 or 13 ppm of SO_2 for 30 min without exhibiting increases in flow resistance beyond the first 10 minutes

Objective: Our objective in these experiments was to determine what effect short-term exposures to controlled concentrations ofSO_2might have on the mechanics of breathing in health subjects.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1, 5, 13 ppm	10-30min	Male	22-56y	11	healthy	No observed adverse effect level	Lowest observed adverse effect level

Observations:

Pulmonary Flow Resistance-variability of measurement
There was great variability among subjects.
Six of the eleven subjects varied significantly in constant values from one experiment to the next.

Dose Response
Nine of eleven subjects showed a significant change in flow resistance after a 10 min exposure to 1 ppm of SO_2. At 5ppm (4-7ppm), flow resistance rose an average of 0.51cm $H_2O/L/sec$ ($p<0.01$), an increase of 39% above control. At 13ppm (range 10-16ppm), the increase was 1.25 cm $H_2O/L/sec$ ($p<0.001$), 82% above control. The change was significantly greater at 13ppm than at 5ppm ($p<0.01$).

Recovery
The average flow resistance for the group still remained elevated 15 min after the end of exposure to both 5 and 13ppm of SO_2. (recovery > control for both concentrations: $p<.01$). At 5ppm this difference was significant in 4 of the subjects, at 13ppm it was not significant for any individual.

Other Measurements

The functional residual capacity showed no consistent trend during exposure to 1 and 5 ppm of SO_2. There was an increase in functional residual capacity at 13 ppm ($P<0.01$), which was not significant. The respiratory rate and tidal volume were unaffected during exposure to the gas.

Symptoms

Symptoms were usually elicited by 4-5 ppm of SO_2 or higher.

*Statistically significant

Author's Conclusions:

"Our experiments show that the MFR (maximum forced expiratory flow rate), measured with the Wright flow meter, may be affected, though only slightly, when PFR (pulmonary flow resistance) increases as little as 1.16 cm $H_2O/L/s$; the changes in TVC (rapid vital capacity) for a smaller group were not significant. There were no consistent changes in pulmonary compliance, tidal volume, breathing frequency, or pulse rate. The functional residual capacity increased slightly during exposure to 13 ppm."

"There is evidence that bronchoconstriction may be mediated by both extrinsic nerve reflexes and direct action of the gas on bronchial smooth muscle. Whether variations in the amount of secretion by the mucosal glands is an additional factor contributing to changes in flow resistance is not certain. If, as in experimental animals, the level of irritant gas is made sufficiently high there is ensuing tissue damage in the form of edema and hemorrhage. Extreme changes of this type may be partially responsible for the pattern of response in those guinea pigs in which there is a continuing rise in flow resistance but they are not likely to have played a role in the present study."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? **(not stated)** Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? **(more info needed)** Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (-) Was the duration of exposure appropriate to investigate the primary objective?

	(-) Was the duration of exposure precisely defined? **(+)** Was the mode of administration appropriate? **(more info needed)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(not stated)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	**(not stated)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined **(-)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(-)** Were the subjects randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(subjects served as own controls)** Was a control group assayed? **(more details needed)** Is the control regime appropriate? **(-)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(-)** Was the length of follow-up appropriate? **(+)** Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(+)** Were appropriate endpoints examined? **(-)** Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(more detail needed)** Were accepted assessment methods employed? **(-)** Were assessment measures sufficiently sensitive? **(-)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(-)(** Was the timing and frequency of assessment logical? **(-)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(some)** Was individual data reported for each subject? **(-)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	**(more detail needed)** Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(more detail needed)** Did the investigator appropriately interpret the statistics? **(few)** Are the observed trends statistically significant?

G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (no clear statements of conclusion) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings this study were inconsistent and mostly non significant. Due to many weaknesses in the study design, particularly with the selection of subjects, the results are of no clinical or toxicological significant.

This study refers only to the effects of brief administration of SO_2 on the respiratory mechanics of healthy male adults. Future studies should consider whether frequent or prolonged exposure to SO_2 might affect the degree of response seen in these experiments.

Confidence index:

The exposure levels and exposure durations varied for each subjects.

There is limited information on how the subjects were recruited. It is mentioned that they volunteered.

Data from 3 additional volunteers are included in some of the analyses; however, there is no information on these subjects.

One of the subjects was an author of the study.

The subjects knew when they were being exposed to SO_2, but not what level.

There was significant variability among the subjects. In some cases results from only a few subjects are reported.

The standard deviations of the exposure levels were very high.

For one of the analyses, data from subjects exposed to SO_2 and SO_2 + saline aerosol are combined. However, there are no other details on where the saline aerosol data came from.

The number of subjects included in the different experiments varied, and no explanation is given on why certain subjects were chosen over others.

Symptom data is only reported for one subject.

The focus of the paper shifts in the discussion and the results are compared to guinea pig data, which was apparently part of this study, although this is not clear.

This study included the rationale for the range of concentrations used. While some attempt was made to limit confounding factors by restricting the study to males only, the study did not employ enough subjects to be able to extrapolate findings to a general population.

This study fails to give details on exposure conditions. Neither the duration nor the level of exposure were precisely defined.

Review – Confidence index ranking:

Low

CLINICAL REVIEW FORM

Author: Sim, V.M., Pattle, R.E.	**Internal ID:** 324
Title:Effect of Possible Smog Irritants on Human Subjects	
Year:1957	

Abstract: Various aerosols and gaseous mixtures were administered to adult male volunteer subjects by two methods, one using a mask, the other involving a chamber large enough for all subjects to occupy at the same time. The main effects of sulfur dioxide gas and sulfuric acid mist inhaled in this way were an increase in airway resistance and appearance of rales, with rhinorrhea and lacrimation. Two subjects exposed to sulfuric acid mist developed long-lasting bronchitic symptoms; the addition of water vapor increased the mean particle-size of the sulfuric acid mist and intensified its irritant effects. These acid substances were neutralized and their irritant effects abolished by adding either ammonia gas or magnesium oxide smoke tot eh atmosphere. While acrolein and crotonaldehyde were highly irritant, formaldehyde was less so; acetaldehyde and its higher homologues were almost nonirritant. It was evident that neither the concentration of sulfur dioxide not the total aldehyde content of an atmosphere is an adequate index of its irritant action, especially on people handicapped by disease or old age.

Objective: The present report details results obtained from the exposure of male volunteer subjects to sulfur dioxide, sulfuric acid mist, and other irritant substances which may be present in a smog atmosphere.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
1.34-80ppm (mask experiments) 1-23.1ppm (exposure chamber experiments)	10 min for each exposure with mask and 60 minutes for each exposure in chamber For the mask experiments data was collected from 264 10min exposures For the chamber experiments data was collected from 330 exposures	Male	18-45	Not stated	Healthy	n/a	Na/

Observations:

In the experiments with sulfur dioxide the concentrations administered by means of the mask varied from 3.16 to 216 mg/m^3 (1.34-80ppm)., with constant exposure times of 10 minutes. The maximum dosage given by mask was 2160 mg.min/m^3 and in the chamber 3620 mg min/m^3. With dosages below 800 mg.min/m^3, little change was noted neither clinically or by measurement of lung resistance to air flow. When dosages above 1330 mg.min/m^3 were used, the lung resistance to air flow was increased significantly above normal in half the people exposed irrespective of whether they received the exposure by mask or in the chamber. The most frequent physical finding at these higher dosages was the presence of high-pitched musical rales, with a tendency to prolongation of the expiratory phase of respiration. Only 8 times in 694 exposures were there significant changes in pulse rate, respiratory rate, volumes of tidal or supplemental air, vital capacity, maximum breathing capacity, or blood pressure. Cold air and SO2 exposures yielded the same results.

*Statistically significant

Author's Conclusions:

The main effects of sulfur dioxide and sulfuric acid were an increase in airway resistance and causation of rales.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(no info) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (no info) Are the subjects a representative sample of the population to which extrapolations will be made? (no info) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (no info) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (more detail needed) Was there a gradient of exposure levels? (more detail needed) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (not stated)Were both nominal and actual exposure concentrations recorded? (-) Was the duration of exposure appropriate to investigate the primary objective? (-) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (more detail needed) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (not stated) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?

C. Equipment:	**(limited info)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined **(-)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(not stated)** Were the subjects randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(-)** Was a control group assayed? **(-)** Is the control regime appropriate? **(no)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(-)** Was the length of follow-up appropriate? **(more detail needed)** Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(-)** Were appropriate endpoints examined? **(-)** Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(more detail needed)** Were accepted assessment methods employed? **(more detail needed)** Were assessment measures sufficiently sensitive? **(-)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(more information needed)** Was the timing and frequency of assessment logical? **(-)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each subject? **(not stated)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	**(no info)** Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(more detail needed)** Did the investigator appropriately interpret the statistics? **(not clearly stated)** Are the observed trends statistically significant?
G. Interpretations:	**(+)** Was the original objective addressed? **(-)** Are the results novel? **(-)** Was the design of the study appropriate to investigate the primary objective? **(-)** Were assumptions reasonable? **(-)** Are the methods reproducible?

	(-) Did either the study design or the statistical analysis limit confounding variables? (-) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The main effects of sulfur dioxide gas were an increase in airway resistance and the appearance of rales, with rhinorrhea and lacrimation. The results were inconsistent and variable among subjects. It is not clear exactly what exposure levels each subject was exposed to and how many exposures occurred for each subject. The toxicological and clinical significance is limited due to a significant lack of information in the study.

A new finding is that adding magnesium oxide smoke or ammonia to an atmosphere containing sulfur dioxide removed the irritant and bronchonstrictive properties of that atmosphere. This study determined that the concentration of SO2 is not an adequate index of its irritant action.

Confidence index:

The sample size is not stated.

The exposure level range is given, but no details on exactly what exposure levels each subject received and how many exposures per subject.

Subjects were allowed to walk around and /or smoke during the exposure if they wished.

There is extremely limited information on the results.

There is no information on how the subjects were examined or tested.

There is no information on the control regime.

There is no information on how the subjects were chosen.

The measurement of SO$_2$was not continuous.

The calculation of some of the effects was not explained.

The exposure duration varied.

Longer exposure times would be more reflective of normal conditions. Future studies should involve a broader spectrum of the community than just healthy young males.

There was no evidence to justify the dosing regime nor were preliminary trials done to establish them.

Review – Confidence index ranking:

Low

CLINICAL REVIEW FORM

Author: Lawther, P.J.	**Internal ID:** 325
Title:Effects of Inhalation of Sulphur Dioxide on Respiration and Pulse-Rate in Normal Subjects	
Year:1955	
Abstract:No Abstract	
Objective: In a study by Amdur, Melvin, and Drinker (1953) it was found that the inhalation of sulphur dioxide in low concentrations produced consistent changes in respiration and pulse-rate. Breathing became shallower and more rapid, and the pulse rate increased. It was decided to examine the effects of sulphur dioxide on the work of breathing, determined by analysis of simultaneous pneumotachograph and esophageal pressure tracings. Initial experiments using virtually the same technique as that of Amdur et al. not only failed to reveal any change in the work of breathing but the expected alterations in respiratory rate and depth were not observed. The objective of the present study is to investigate the matter by conducting more fully experiments under rigorously controlled conditions.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0, 5, 10, 20ppm	10 min each	Males	21-40y	N=18 (Four experiments and each experiment used 6 subjects) For the experiments with 20ppm, n=6 (from the original 18)	healthy	n/a	n/a

Observations:

If all the groups are considered together, the changes in pulse rate, respiratory rate, and tidal volume, observed during and after the inhalation of 5 or 10 ppm are very small. There is no regular variation with gas concentration. Results are consistent with a hypothesis of no change in the quantities measured during "exposure" and "recovery" periods, compared with "basal" values at any of the concentrations 0ppm, 5ppm and 10ppm of sulphur dioxide. Differences between the results in the nose breathing and mouth breathing groups and between those in town and country groups are not significant at the 5% level. Six volunteers

were given 20ppm and no consistent effects on the values measured were noted. Examination of individual results in all the groups shows no consistent change, and no subject showed the triad of effects reported in previous studies.
*Statistically significant

Author's Conclusions:

"We have been unable to confirm the findings of Amdur et al…it is considered likely that the consistent nature of the findings of Amdur et al. were due to chance in a small series of observations. The results reported here only indicate that the inhalation of sulphur dioxide produces no consistent change in respiratory rate, tidal volume, or pulse-rate. It should be realized, however, that susceptible individuals might react in a characteristic manner."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? **(no info)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? **(no info)**Were subjects informed about the experimental risks? Was this information provided in such a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (-) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(not stated)** Were both nominal and actual exposure concentrations recorded? (-) Was the duration of exposure appropriate to investigate the primary objective? (-) Was the duration of exposure precisely defined? **(more info needed)** Was the mode of administration appropriate? **(more info needed)** Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? **(no info)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?

D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? (+) Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(subjects served as own controls)** Was a control group assayed? **(n/a)** Is the control regime appropriate? **(n/a no blinding)**Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(-)** Was the length of follow-up appropriate? **(more info needed)** Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(more info needed, no detail)** Were appropriate endpoints examined? **(-)** Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(more detail needed)** Were accepted assessment methods employed? **(-)** Were assessment measures sufficiently sensitive? **(-)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(more info needed)** Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each subject? **(+)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	**(no info on stat analysis)** Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(n/a)** Did the investigator appropriately interpret the statistics? **(no info)** Are the observed trends statistically significant?
G. Interpretations:	**(+)** Was the original objective addressed? **(-)** Are the results novel? **(-)** Was the design of the study appropriate to investigate the primary objective? **(-)** Were assumptions reasonable? **(-)** Are the methods reproducible? **(-)** Did either the study design or the statistical analysis limit confounding variables? **(-)** Do the conclusions make biological sense? **(no extrapolations)** Are the extrapolations reasonable? +) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

No consistent changes in the tidal volume, respirator rate, or pulse rate followed the inhalations for 10 min of 5ppm and 10 ppm of SO_2 . Clinical significance cannot be determined from these results. The results were too poorly presented and too variable among the subjects.

This study was intended to replicate a similar experiment previously performed but to do so under rigorously controlled conditions. The author had previously attempted to replicate the studies but had failed to achieve the same results. In this experiment, the authors attempted to account for any problems with the initial study. Unfortunately, this study produced very few significant findings.

Confidence index:

The study is very poorly designed and needs more detail in the methods and results section.

There is limited information on the subjects or recruitment of subjects.

The results are inconsistent for SO_2 exposure.

The methods are lacking serious detail. In one instance, the authors state that to compare the urban subjects to rural subjects, the apparatus was taken to a rural site and experiments were made on country dwellers (volunteers from a research station). There is insufficient detail presented. These subjects would have been exposed to the experiments under different conditions.

More information is needed on which subjects took part in which experiments. Some subjects took part in more than one experiment.

When a subject's responses were inadequate in one instance, another subject was substituted into the experiment. There is insufficient detail on the new subject.

In another experiment, the tests are performed on a psychologist who wanted to volunteer out of interest. So, in this case, n=1.

Data/results were only given in percentages.

Review – Confidence index ranking:

Low

CLINICAL REVIEW FORM

Author: Bethel, R.A., Erle, D.J., Epstein, J., Sheppard, D., Nadel, J.A., Boushey, H.A.	**Internal ID:** 326

Title:Effect of Exercise Rate and Route of Inhalation on Sulfur-Dioxide induced Bronchoconstriction in Asthmatic Subjects

Year:1983

Abstract: Nine asthmatic subjects exercised at low, moderate, and high work rates on a cycle ergometer while breathing filtered, humidified air with or without 0.5 ppm of sulfur dioxide (SO_2) in a double-blind study. Subjects first performed these experiments breathing through a mouthpiece while wearing a noseclip (oral breathing) and then repeated the experiments breathing through a facemask that separated and permitted independent measurement of oral and nasal air flow (oronasal breathing). We determined specific airway resistance before and after exercise by body plethysmography. Inhaled by mouthpiece, 0.5 ppm SO_2 caused bronchoconstriction at moderate and high but not at low work rates. There was a dose-response relationship between the work rate performed and the degree of bronchoconstriction induced. Inhaled oronasally, 0.5 ppm SO_2 caused bronchoconstriction only at the high work rate. These findings demonstrate that SO_2-induced bronchoconstriction is dependent on the work rate of exercise during exposure that oronasal breathing is only partially effective in preventing the bronchoconstriction observed with oral breathing, and that oronasal breathing is less effective in preventing bronchoconstriction with high than with moderate exercise at this concentration of SO_2.

Objective: The purposes of this study were to determine the influence of work rate on the bronchoconstriction induced by SO_2 and to determine whether the degree of protection given by oronasal breathing against SO_2induced bronchoconstriction is depend on work rate.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
0.5ppm SO_2	5 minutes	6 women 3 men	20-37y	9	Nonsmoking Asthmatic	No observed adverse effect level	Lowest observed adverse effect level

Observations:

In experiments in which subjects breathed through a mouthpiece, 0.5ppm SO_2 did not cause bronchoconstriction during light exercise but did cause bronchoconstriction during moderate exercise ($p<0.001$) and an even greater degree of bronchoconstriction during heavy exercise ($p<0.001$). The degree of bronchoconstriction induced by SO_2during moderate and heavy exercise was not significantly different.

In experiments in which subjects breathed through a face mask, sulfur dioxide did not cause bronchoconstriction during light or moderate exercise but did cause bronchoconstriction during heavy exercise ($p<0.001$). At high exercise work rates, oronasal breathing diminished but did not prevent the bronchoconstriction induced by SO_2 in mouthpiece experiments.

Although the proportion of total ventilation breathed by mouth was greater during heavy than during light or moderate exercise, this difference was not statistically significant.

There was a dose-response relationship between exercise work rate performed and the mean bronchoconstriction induced by SO_2. In experiments in which SO_2 was administered oronasally, SO_2 did not cause bronchoconstriction during light or moderate exercise but did cause bronchoconstriction during heavy exercise. During face mask experiments, both oral and nasal ventilation increased incrementally as exercise work rate increased.

*Statistically significant

Author's Conclusions:

These findings demonstrate that SO_2-induced bronchoconstriction is dependent on the work rate of exercise during exposure, that oronasal breathing is only partially effective in preventing the bronchoconstriction observed with oral breathing, and that oronasal breathing is less effective in preventing bronchoconstriction with high than with moderate exercise at this concentration of SO_2.

"The distribution of airflow between the nose and the mouth, as subjects increased their exercise work rate, varied greatly among subjects. We had predicted that with increasing work rate, subjects would breathe a greater proportion of their total ventilation through the mouth. A consequence of this predicted change would be that proportionately more SO_2 would reach the lungs. Regardless of the relative proportions of ventilation through the nose and the mouth, absolute ventilation through both the nose and the mouth increased incrementally with increase in exercise work rate in almost all instances. Thus as exercise increased, a greater dose of SO_2 was delivered to the larynx and lower airways through both the nose and the mouth."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+) **except work history** Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (**(more info needed on selection)** Are the subjects a representative sample of the population to which extrapolations will be made? **(more info needed)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? **not stated** Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective?

	(+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Were the subjects randomly assigned to exposure groups? **(no info)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(subjects served as own controls)** Was a control group assayed? (+) Is the control regime appropriate? **(double)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (not stated) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **((n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?

G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) **more detail needed** Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings in this study demonstrated that the degree of bronchoconstriction induced by SO_2 in subjects with asthma depends on the work rate of exercise performed as they breathe SO_2. Higher work rates result in greater bronchoconstriction, presumably because of the increased rate at which the dose of SO_2 is delivered to the lungs with increased ventilation. A dose-response relationship was observed between work rate and bronchoconstriction in persons with asthma; however, the findings in the study were variable between the subjects. For example, the degree of bronchoconstriction induced by SO_2 was highly variable. The pattern of oral and nasal distribution of ventilation also differed greatly among subjects.

Results from this study confirm findings from previous studies that showed that oronasal breathing only partially prevents the bronchoconstriction induced by SO_2. Nasal or oronasal breathing is less effective at preventing SO2-induced bronchoconstriction during heavy than during moderate exercise.

Confidence index:

This study was well designed.

More information is needed on the selection of the subjects. Two of the subjects were from previous experiments by the same authors.

The methods section is missing some information, but otherwise is very detailed.

The results between the subjects were quite variable. The facemask may have altered the oronasal distribution of breathing during exercise. According to the authors, the facemask, which fitted snugly about the nose and mouth, may have caused subjects to breath oronasally at exercise work rates at which they would have breathed only nasally during free breathing. The resultant increase in oral ventilation would have increased the dose of SO_2 to the lungs causing greater degrees of bronchoconstriction. Or, the facemask might have prevented a subject from opening his mouth as widely as he would have during free breathing.

There was only one exposure level.

Review – Confidence index ranking:

Moderate

CASE-CONTROL REVIEW FORM

Author: **Ito, K., Thurston, G.D., Hayes, C., Lippman, M.** **Internal ID:** 330

Title:

Associations of London, England, Daily Mortality with Particulate Matter, Sulfur Dioxide and Acidic Aeorosol Pollution

Year:1993

Abstract:

During the extreme pollution episodes of 1952 and 1962 in London, England, excesses in daily mortality were clearly evident. In this study, we examined daily British Smoke, sulfur dioxide, acid aerosols, and weather variables for their short-term associations with daily mortality in the more typical (nonepisodic) winters of 1965-1972. Consideration of the acid aerosol data was of special interest because this chemical component has been suspected as a causal agent in past episodes. Temporal lag structures between the variables were examined after removal of long-term components from each series in order to obtain "rational" cross-correlations. Significant associations between same-day and lagged pollution variables and mortality were found. Alternative regression models with pollution and weather variables were also developed. The coefficients obtained were applied to the 1962 pollution episode to examine the continuity of the estimated slopes. The pollution-predicted deaths fit the observed deaths well, which supports the applicability of such deviation-derived coefficients to the absolute scale. These models were also employed to estimate mean excess daily deaths attributed hypothetically to air pollution. On average, mean effect ranged from 2-7% of all deaths during the nonepisodic winters in Greater London, but the 95% confidence intervals of these estimates overlapped for all model specifications examined. This estimated pollutant mixture "effect" cannot be attributed to a particular pollutant because of a lack of quantitative information on the relative downward biases caused by both analytical errors and errors in the spatial representativeness of each respective pollution index.

Objective: The primary goal of the research reported in this paper was to utilize time series methods to evaluate the associations between each of the three pollutants studied and daily mortality. These models were employed to estimate a range of mortality "effects", if any, attributable to air pollution when each measured pollutant was used as an index of the London air pollution mixture. Finally, the regression coefficients obtained from the non episodic period were applied to the 1962 episode data to examine the continuity of the hypothetical mortality/pollution slope.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Up to 1000μg/m^3	1965-1972	No info	No info	No info	n/a	n/a	n/a

Observations:

Significant associations between same-day and lagged SO_2 and mortality were found. Alternative regression models with pollution and weather variables were also developed. The coefficients obtained were applied to the 1962 pollution episode to examine the continuity of the estimated slopes. The pollution-predicted deaths fit the observed deaths well, which supports the applicability of such deviation-derived coefficients to the absolute scale. These models were also employed to estimate mean excess daily deaths attributed hypothetically to air pollution. On average, mean effect ranged from 2-7% of all deaths during the nonepisodic winters in Greater London, but the 95% confidence intervals of these estimates overlapped for all model specifications examined.

*Statistically significant

Author's Conclusions:

"This estimated pollutant mixture "effect" cannot be attributed to a particular pollutant because of a lack of quantitative information on the relative downward biases caused by both analytical errors and errors in the spatial representativeness of each respective pollution index."

"All pollutants measured daily were found to be associated significantly with mortality effects. It is not appropriate, however, to interpret the small (nonsignificant) differences in strength of association among the three pollutants considered as indicative of their respective importance to mortality. This is because of the pollutants collinearity and the lack of quantitative information on their relative measurement errors."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? **(details in another study)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	**(details in another study)** Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(details in another study)** Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls?

	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) **mortality** Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (not stated) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (some) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? **(n/a no extrapolations)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study was designed to look at how different statistical models will produce different conclusions. How prefiltering of the data influences the subsequent health effects analyses. The study presented a very thorough and informative paper on modeling air pollution data and discusses the many errors that may affect the observance of associations. Regression coefficients were calculated and used to predict the expected number of deaths during the pollution episodes. The pollution predicted deaths fit the observed deaths well.

This study attempted to extrapolate a generalized exposure/human health response relationship to air pollution. While this type of study has been done before, previous experiments have produced different conclusions regarding the effects of specific pollutants on human health. This study points out that studies could use error estimates during the design phase to minimize statistical bias.

Confidence index:

Most of the method information is included in another study. However, the main objective is to look at how manipulating the data and using different models affects the associations. The study details methods of statistical analysis and fully describes cross-correlation function analysis/regression.

Review – Confidence index ranking:

Moderate

CASE-CONTROL REVIEW FORM

Author: Moolgavkar, S.H., Luebeck, E.G., Anderson, E.L. | **Internal ID:** 331

Title:
Air Pollution and Hospital Admissions for Respiratory Causes in Minneapolis-St. Paul and Birmingham

Year:1997

Abstract:

We investigated the association between air pollution and hospital admissions for chronic obstructive pulmonary disease and pneumonia among the elderly in Minneapolis-St. Paul, MN, and Birmingham, AL, over the period January 1, 1986, to December 31, 1991. Pollutants included in our analyses were PM_{10} (particulate matter less than 10 microns in aerodynamic diameter), SO_2, NO_2, O_3, and CO in Minneapolis-St. Paul, and PM_{10}, O_3, and CO in Birmingham. After adjusting for temperature, day of week, season, and temporal trends, we found little evidence of association between air pollution and hospital admissions for respiratory causes in Birmingham. In contrast, we found that air pollution was associated with hospital admissions for respiratory causes in Minneapolis-St. Paul. Among the individual pollutants, O_3 was most strongly associated with admissions (estimated increase in hospital admissions associated with a 15-parts-per-billion increase in O_3 on the previous day = 5.15%; 95% confidence interval = 2.36-7.94%), and this association was robust in the sense that it was little affected by the simultaneous consideration of other pollutants. PM_{10}, SO_2, and NO_2 were also associated with hospital admissions, although none could be singled out as being more important than the others.

Objective: In this study, we undertook analyses of air pollution and daily hospital admissions for respiratory causes in the cities of Birmingham, AL, and Minneapolis-St.Paul, MN, during the period 1986-1991.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Minneapolis-St.Paul:4.82 ppb (2191 days) Birmingham:6.58ppb (1307days)	1986-1991	No info	65 y and older	Respiratory Mortality: Minneapolis:10.55/day Birmingham:8.26/day	No info	n/a	n/a

Observations:

SO_2 analysis was not done for Birmingham due to the amount of missing information.
In Minneapolis, each of the pollutants, with the exception of CO, was associated with total respiratory hospital admissions when considered alone in the basic regression model. Among PM_{10}, SO_2, and NO_2, it was not possible to single out one as being more important than the others.
The estimated effect for SO_2, reported as the percentage change in hospital admissions associated with an increase in SO_2 level approximately equal the interquartile range (3.5ppb) was 1.6% (95% CI -0.1%-3.3%), lag 2 days.

*Statistically significant

Author's Conclusions:

"In conclusion, there was clearly an association between air pollution and daily hospital admissions for respiratory diseases in Minneapolis-St. Paul. Although PM_{10}, SO_2 and NO_2 were positively associated with hospital admissions, no single one of this group of pollutants stands out as being more important than the others. Our analyses in Minneapolis showed positive associations between individual pollutants and hospitals admissions in regression models, even with multiple pollutants in the model and when concentrations of some pollutants were quite low. The appropriate interpretation of this finding is that daily fluctuations in the concentration of the pollutant reflected fluctuations in the air pollution complex associated with hospital admissions."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ((+) over 65y Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(+) Was a full description of the monitoring devices provided? (not stated) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)

E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (n/a no symptom data) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (no extrapolations) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings: Positive associations were observed between $SO_2$and hospital admissions for COPD; however, these associations were not statistically significant. The SO_2analyses could only be performed for one city due to the large amount of missing data in Birmingham, preventing comparison.

This study attempts to examine the effect of air pollution as a whole, rather than a sum of its components, on health. The author points out that results of regression analysis that consider only a few of the criteria pollutants are even more difficult to interpret because the possibility of confounding by the omitted pollutants cannot be ruled out. The findings involve O_3, PM_{10}, SO_2 and NO_2, but are somewhat vague with respect to SO_2, finding only that PM_{10}, SO_2, and NO_2

may be associated with hospital admissions, although none could be singled out as being more important than the others.
There was clearly an association between air pollution and daily hospital admissions for respiratory diseases. This paper says that if further progress is to be made, the focus must shift from individual pollutants to the consideration of air pollution as a complex mixture.

Confidence index:
Most confounders are considered. Influenza epidemics are not mentioned.
There is practically no information on the monitoring stations, including their location and the number of monitors..
It is not stated if residence information was available from the admissions data.
The confidence intervals were wide.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Mazumdar, S., Schimmel, H., Higgins, I.T.T.	**Internal ID:** 332
Title: **Relation of Daily Mortality to Air Pollution: An Analysis of 14 London Winters, 1958/59-1971/72**	
Year:1982	
Abstract: The relationship between daily deaths and daily concentrations of Smoke and SO_2 in London, England for 14 winters during the years 1958-1959 through 1971-1972 has been explored. Three types of analyses were used: (1) year-by-year multiple regression; (2) stratification using nested quartiles of one pollutant within quartiles of the other; and (3) multiple regression of a special subset of high pollution days. An association was found with Smoke, but not with SO_2. Whether a linear model with zero threshold or a threshold model best fits could not be determined unambiguously because of a statistical artifact. Reasons for preferring a threshold-type quadratic model are given.	
Objective: In addition to confirming the adverse effects of high pollution, our present study of London data for the 14 winters of 1958-1959 through 1971-1972 has differentiated between the possible separate influences associated with$SO_2$and Smoke measures.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Range of winter means: $0.181 mg/m^3$-$0.419 mg/m^3$ First 7 winters: $0.334 mg/m^3$ Second 7 winters: $0.215 mg/m^3$ All winters:$0.275 mg/m^3$	14 winters 1958-1972	No info	No info	Average daily number of deaths for all winters:292/day	No info	n/a	n/a

Observations:

Analysis 1. Separate multiple regressions for each winter
We maintain that the 14 winter average of SO_2 coefficients is trivial compared to that of smoke, being only 1.165% change in mortality per mg/m^3 of SO_2 The

results for SO_2 were not significant.

Analysis 2: Stratification using two way nested quartiles
Quantitative estimates of percent change in mortality per mg/m^3 were calculated by combining estimates from individual quartiles. The results are 6.16% for SO_2 per mg/m^3.

Analysis 3: Multiple regression study of high pollution data set.
During 14 yr there were 100 days when pollution was in the highest decile of each pollutant. As SO_2increases from 0.4 to approximately $0.7mg/m^3$, mortality response declines and above the 0.7 level mortality gradually increases.
There may be SO_2/smoke synergism even though their crossproduct was not significant in the initial regression.

*Statistically significant

Author's Conclusions:

An association was not found with SO_2.

"Our analyses of London data for 14 winters support the conclusion that mortality is positively associated with air pollution. Our much larger data base and three different methods of analysis also permit the additional conclusion that the pollution/mortality association is due almost entirely to smoke."

"Analysis of the high pollution data set suggested a possible contribution of SO_2 at sufficiently high SO_2 and smoke levels (both are > $0.8\ mg/m^3$). The result, however, is ambiguous since the high pollution data set equation predicts a mortality excess of 185% for the peak SO_2 day of the entire set, when the actual response is a little over 30%. This value is predicted closely by does-response curves based on Smoke alone and $(Smoke)^2$ alone."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (not stated) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval?

	(n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? **(no extrap.)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The associations observed in this study between $SO_2$and mortality were not statistically significant and the magnitude of the effects were small. Analysis of the high pollution data set suggested a possible contribution of SO_2 at sufficiently high levels (>0.8mg/m^3). The result, however, is ambiguous since the high pollution data set equation predicts a mortality excess of 185% for the peak SO_2 day of the entire data set (SO_2 =3.41mg/m^3), when the actual response is a little over 30%.

An association was found between daily deaths and smoke but not between daily deaths and SO_2. That said, this study talks mainly about smoke and few conclusions were made on SO_2.

Confidence index:

The introduction is very brief.

There is limited justification given for the different methods used to analyze the data.

Because the data was collected for previous studies, none of the methods, monitoring information etc. are included in this study.

Some of the assumptions of the models are questionable. For example, the assumption of a linear relationship between mortality and pollution.

This study did not sufficiently study possible confounding factors such as the nonlinear functions of weather. No demographics of the population being used were described.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Moseholm, L., Taudorf, E., Frosig, A.	**Internal ID:** 333

Title:

Pulmonary Function changes in Asthmatics Associated with Low-Level SO_2 and NO_2 air pollution, weather, and medicine intake

Year:1993

Abstract: This paper presents the response in subjects with asthma to gaseous air-pollution levels, weather, and medicine intake as identified by principal-component analysis and neural network techniques. Pulmonary function measured by respiratory peak-flow rate in nonallergic asthmatics was associated with ambient, low-level, air-pollution concentrations of sulfur dioxide and nitrogen dioxide, temperature, relative humidity, and medicine intake. Results from 27 nonallergic asthmatics aged 18-60 years with well-characterized bronchial asthma and regular medical treatment were analyzed from two cities. During an 8-month period, each subject kept a diary table, which included symptoms, lung function (evening peak flow), medicine intake, and tobacco smoking. High intake of medicine and high ambient temperatures corresponded to decreased peak flow. The changes in temperature did not occur in situations with low medicine intake. During frost periods, peak-flow values decreased independently of medicine intake and levels of SO_2. During other times, increased levels of SO_2 and NO_2 increased temperature, and increased intake of medicine, and low relative humidity corresponded to decreased peak flow. Increased levels of SO_2 and NO_2 corresponded synergistically to decreased peak flow at levels above 40 micrograms/m^3.

Objective: A prospective open study was initiated at five Danish hospitals in four cities to identify possible relationships between the relatively low Danish air pollution levels and meteorological parameters and illness in a population of nonallergic asthmatics.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
24hr mean range: 15-18µg/m^3	8 months	No info	18-60	73	asthmatic	n/a	n/a

Observations:

Pulmonary function measured by respiratory peak-flow rate in nonallergic asthmatics was associated with ambient, low-level, air-pollution concentrations of sulfur dioxide, temperature, relative humidity, and medicine intake. High intake of medicine and high ambient temperatures corresponded to decreased peak flow. During frost periods, peak-flow values decreased independently of medicine intake and levels of SO_2. During other times, increased levels of SO_2 increased temperature, and increased intake of medicine, and low relative humidity corresponded to decreased peak flow. Increased levels of SO_2 corresponded synergistically to decreased peak flow at levels above 40 mg/m^3.

Author's Conclusions:

Our results showed that the neural network's efficiency at nonlinear fitting, and its learning ability , i.e. ability to ignore contradictory results and outliers, allowed it to generalize the generic pattern to the same extent as, or to a greater extent than, the classic methods. However, the network makes it possible to exposure a far greater range of potential solutions to the dose response function than do conventional techniques.

"We found that increased levels of SO_2 and NO_2 correspond synergistically to decreased peak flow at levels above 40 ug/m^3, and that effects of the pollutants depend nonlinearly on the characteristic weather. The complexity of the many parameters studied, including the qualitative nature of some variables and the multiple nonlinear relationships, makes it difficult to apply classical regression techniques."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (more info needed) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (not stated) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (not stated) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) not stated Was the nature and severity of symptoms graded against an explicitly defined scale? (not stated) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (some) Was raw data provided to permit the reader to arrive at his/her own conclusions?

	(-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (more info needed) Did the investigator appropriately interpret the statistics? (not stated) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? more detail needed ere assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Due to the lack of understanding of the possible dose-response relationships between SO_2 and human health, neural networks were applied to recognize patterns in asthmatic responses. In the past, neural networks have been shown to provide efficient solutions to problems that are difficult to solve by traditional statistical techniques. This study aims to use neural networks as a means of explaining patterns in asthmatic responses, in that case, to SO_2. This study is novel and relevant in that it has recently been concluded that neural networks will likely make a significant contribution to clinical practice. On the other hand, the results may be more difficult to evaluate because of the lack of the usual F or t-test for statistical significance.

Confidence index:

More detail is needed on the test subjects.

The subjects are self selected.

There is very limited information on the monitoring.

Only 2 of the 4 cities had SO_2data, and in these 2 cities, there was only one central monitor each.

There is no mention of the consideration of a training effect with the peak flow meter.

Overall, the study provided good detail on the model used and presents a unique and novel method for analyzing this data.

The authors made an effort to select cities that were similar in size with mixed industry, to limit confounding factors.

Much of the data are extracted from a subject kept diary table and no information on equipment used was given.

Review – Confidence index ranking:

Moderate to low

CASE-CONTROL REVIEW FORM

Author: Moolgavkar, S.H., Luebeck, E.G., Hall, T.A., Anderson, E.L.	**Internal ID:** 334
Title: **Air Pollution and Daily Mortality in Philadelphia**	
Year:1995	
Abstract: Many recent analyses have reported associations between air pollution and mortality in U.S. cities. In this paper, we present the results of regression analyses of daily mortality in Philadelphia during the period 1973-1988. Pollution variables included in the analyses were total suspended particulates (TSP), sulfur dioxide, and ozone. We controlled for the effects of weather on mortality by analyzing mortality separately for each season and explicitly including quintiles of temperature in the regression models. In regression models that consider weather and pollution variables simultaneously, daily mortality is associated with hot days in summer [relative risk (RR) for highest quintile of temperature = 1.07; 95% confidence interval (CI) = 1.04-1.10], and with cold days in spring (RR for lowest quintile of temperature = 1.07; 95% CI = 1.04-1.10), fall (RR for lowest quintile of temperature = 1.05; 95% CI = 1.02-1.08), and winter (RR for lowest quintile of temperature = 1.04; 95% CI = 1.01-1.07). When all three pollution covariates and weather are considered simultaneously in the regression model, ozone is associated with mortality in summer (RR = 1.15; 95% CI = 1.07-1.24) and sulfur dioxide is associated with mortality in spring (RR = 1.19; 95% CI = 1.06-1.33), fall (RR = 1.14; 95% CI = 1.00-1.29), and winter (RR = 1.21; 95% CI = 1.09-1.35), where the relative risks are for incremental changes of 100 parts per billion in ozone and sulfur dioxide on the previous day. Because the pollution covariates are highly correlated, it is premature to single out one specific component as being responsible for the observed association between air pollution and mortality.	
Objective: In this paper, we report the results of our analyses of the daily mortality in Philadelphia during the years 1973-1988 inclusive.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Daily Mean: Spring-16.8ppb Summer-15.7ppb Fall-17.8ppb Winter-25.4ppb	1973-1988	No info	No info	Daily deaths (variance) : Spring-54.4(75) Summer-51.0 (78.3) Fall-52.6 (66.5) Winter-59.3 (97.4)	No info	n/a	n/a

Observations:

The relative risks were calculated for 100ppb increase of SO_2. SO_2 was positively associated with mortality in each season. The relative risks are all between 1.02-1.10.

When all three pollution covariates and weather are considered simultaneously in the regression model sulfur dioxide is associated with mortality in spring (RR = 1.19; 95% CI = 1.06-1.33), fall (RR = 1.14; 95% CI = 1.00-1.29), and winter (RR = 1.21; 95% CI = 1.09-1.35), where the relative risks are for incremental changes of 100 parts per billion in sulfur dioxide on the previous day.
In regression models that consider weather and pollution variables simultaneously, daily mortality is associated with hot days in summer and with cold days in spring, fall, and winter.

*Statistically significant

Author's Conclusions:

"Because the pollution covariates are highly correlated, it is premature to single out one specific component as being responsible for the observed association between air pollution and mortality."
"We believe that the focus on particulates has not allowed for adequate control of other pollutants. Clearly air pollution is a complex mixture. In our current state of knowledge, a specific component of air pollution cannot be singled out as being responsible for association between air pollution and mortality. The Philadelphia data are certainly consistent with the hypothesis that different components of pollution are associated with mortality in different seasons. Because of the high correlation among the various components of air pollution, however, synergistic effects cannot be ruled out."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (not stated) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate?

	(+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (n/a no symptom data) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (some) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (not stated) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (more detail needed) Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (no extrapl) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Positive associations were observed between SO_2 and mortality. The magnitude of the effects were small.

This study was not novel in that two previous analyses of air pollution and daily mortality in Philadelphia have previously appeared in the literature. However, this study suggests that while daily pollution does appear to be associated with daily mortality, it is premature to single out a particular component of pollution as being responsible.

This paper considered multiple pollutants and data pertaining to SO_2 was very limited. This paper states that if further progress is to be made, the focus must shift from individual pollutants to the consideration of air pollution as a complex mixture.

Confidence index:

There is limited information on the monitoring system.

Influenza epidemics are not considered.

Some of the comparisons to other studies that analyzed the same data are interesting. Using different models, the results appear inconsistent across the studies.

Missing data were ignored.

The authors also pointed out, that depending on how the SO_2 data are averaged, the results of the associations will change.

This paper controlled for the effects of weather by using Poisson regression and considering the copollutant sulfur dioxide. Results indicated that the introduction of the second pollution covariate into the regression model containing one of the two covariates attenuated the effect of the first. The data did not indicate autocorrelation among the residuals. Consequently, the authors felt it was unnecessary to employ statistical methods to address auto-correlation in the data.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Katsouyanni, K., Touloumi, G., Spix, C., Schwartz, J., Balducci, F., Medina, S., Rossi, G., Wojtyniak, B., Sunyer, J., Bacharova, L., Schouten, J.P., Ponka, A., Anderson, H.R.	**Internal ID:** 336
Title: Short term effects of ambient sulphur dioxide and particulate matter on mortality in 12 European cities: results from time series data from the APHEA project.	
Year:1997	
Abstract: OBJECTIVES: To carry out a prospective combined quantitative analysis of the associations between all cause mortality and ambient particulate matter and sulphur dioxide. DESIGN: Analysis of time series data on daily number of deaths from all causes and concentrations of sulphur dioxide and particulate matter (measured as black smoke or particles smaller than 10 microns in diameter (PM_{10})) and potential confounders. SETTING: 12 European cities in the APHEA project (Air Pollution and Health: a European Approach). MAIN OUTCOME MEASURE: Relative risk of death. RESULTS: In western European cities it was found that an increase of 50 micrograms/m^3 in sulphur dioxide or black smoke was associated with a 3% (95% confidence interval 2% to 4%) increase in daily mortality and the corresponding figure for PM_{10} was 2% (1% to 3%). In central eastern European cities the increase in mortality associated with a 50 micrograms/m^3 change in sulphur dioxide was 0.8% (-0.1% to 2.4%) and in black smoke 0.6% (0.1% to 1.1%). Cumulative effects of prolonged (two to four days) exposure to air pollutants resulted in estimates comparable with the one day effects. The effects of both pollutants were stronger during the summer and were mutually independent. CONCLUSIONS: The internal consistency of the results in western European cities with wide differences in climate and environmental conditions suggest that these associations may be causal. The long term health impact of these effects is uncertain, but today's relatively low levels of sulphur dioxide and particles still have detectable short term effects on health and further reductions in air pollution are advisable.	
Objective: To carry out a prospective combined quantitative analysis of the associations between all cause mortality and ambient particulate matter and sulphur dioxide. DESIGN: Analysis of time series data on daily number of deaths from all causes and concentrations of sulphur dioxide and particulate matter (measured as black smoke or particles smaller than 10 microns in diameter (PM_{10})) and potential confounders.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Median (Range for 12 cities) : 13-74 μg/m^3 Mean winter SO_2: 30-330μg/m^3	Varied for each city 5-14 years	No info	No info	12 cities Number of deaths/day: 8-199	No info	n/a	n/a

Observations:

Mean winter concentrations of SO_2 varied from 30 to 330 μg/m^3. Pooled relative risks were calculated for a 50 μg/m^3 increase in SO_2. Overall, an increase of 50

$\mu g/m^3$ in the one day pollutant levels was associated with an increase in the daily mortality of 3% in the western European Cities for SO_2 or black smoke. The corresponding figures for central eastern cities were 1% for SO_2. Significant heterogeneity was found for the effects of sulphur dioxide.
The participating cities showed substantial variation in air pollution mixtures and concentrations and in geographical distribution, seasonal patterns, and meteorological and climatic conditions. All pollutants analysed had highest concentrations during winter. The effects of SO_2 and black smoke were similar for days with low or high levels of the other pollutant and the same as their overall effect.

*Statistically significant

Author's Conclusions:

In conclusion, the consistency of results from the western European cities with wide differences in topography, climate, environment, and air pollution sources supports a causal association between exposure to particulate matter and sulphur dioxide and mortality from all causes. The reported relative risks are small, but the short term effects of air pollution are not a trivial public heath problem if the ubiquity of air pollution exposure is taken into account.
"The internal consistency of the results in western European cities with wide differences in climate and environmental conditions suggest that these associations may be causal. The long term health impact of these effects is uncertain, but today's relatively low levels of sulphur dioxide and particles still have detectable short term effects on health and further reductions in air pollution are advisable."
"Sulphur dioxide is a highly reactive gas with short half life indoors. It is a known respiratory irritant and bronchoconstrictor, but its effects seem limited to patients with asthma and bronchitis, although sensitivity to exposure varies widely. Exposure to sulphur dioxide may therefore not completely explain the observed increase in mortality; it may rather serve as a surrogate of other substances. Since sulphur dioxide is highly correlated with the levels of fine particles in some American cities, Schwartz et al. postulated that sulphur dioxide may be a marker of fine particles. However, the fact that we found a consistently significant effect of sulphur dioxide in all European cities, whatever the level and composition of particles in each one, may suggest that sulphur dioxide has a direct effect. The role of outdoor peak exposures to sulphur dioxide in the increase of daily mortality should be further investigated."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (n/a) metaanalysis Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitors Was the exposure concentration measured? (+) varied for each city Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?

D. Procedural:	**(n/a metaanalysis)** Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (n/a metaanalysis) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **((n/a metaanalysis** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) **for each city** Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? **(yes)** Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There was a significant effect of moderate to low levels of sulphur dioxide in western European cities. The results for the eastern cities were not significant. The results for all of the cities combined were significant. The meta analysis showed that increased in $SO_2$are associated with increase total mortality and provide evidence that air pollution below guideline levels may have adverse short-term health effects.

The findings for this study support a causal relationship between exposure to sulfur dioxide and mortality. The author points out that while the risks are small, the short-term health effects of air pollution should not be considered trivial if the ubiquity of air pollution exposure is considered. These results support findings from a previous project that found a relationship between sulfur dioxide and cardiovascular and respiratory mortality.

Confidence index:

All of the studies included in the meta analysis were part of the APHEA project. Therefore there were standardized methods for data collection, confounders, statistical methods etc. Potential confounders accounted for included: daily temperature, humidity, day of the week and influenza epidemics, choosing the best air pollution models and applying diagnostic tools to check the adequacy of the models.

There was substantial variation between the cities in air pollution mixtures and concentrations and in geographical distribution, seasonal patterns, and meteorological and climatic conditions.

It was not mentioned in this study as to where the environmental data came from or what monitoring devices were used.

Review – Confidence index ranking:

Moderate

CASE-CONTROL REVIEW FORM

Author: Spix, C., Heinrich, J., Dockery, D., Schwartz, J., Volksch, G., Schwinkowski, K., Collen, C., Wichmann, H.E.

Internal ID: 337

Title:

Air Pollution and Daily Mortality in Erfurt, East Germany, 1980-1989

Year:1993

Abstract: In Erfurt, Germany, unfavorable geography and emissions from coal burning lead to very high ambient pollution (up to about 4000 micrograms/m^3 SO_2 in 1980-89). To assess possible health effects of these exposures, total daily mortality was obtained for this same period. A multivariate model was fitted, including corrections for long-term fluctuations, influenza epidemics, and meteorology, before analyzing the effect of pollution. The best fit for pollution was obtained for log (SO_2 daily mean) with a lag of 2 days. Daily mortality increased by 10% for an increase in SO_2 from 23 to 929 micrograms/m^3 (5% quantile to 95% quantile). A harvesting effect (fewer people die on a given day if more deaths occurred in the last 15 days) may modify this by +/- 2%. The effect for particulates (SP, 1988-89 only) was stronger than the effect of SO_2. Log SP (daily mean) increasing from 15 micrograms/m^3 to 331 micrograms/m^3 (5% quantile to 95% quantile) was associated with a 22% increase in mortality. Depending on harvesting, the observable effect may lie between 14% and 27%. There is no indication of a threshold or synergism. The effects of air pollution are smaller than the effects of influenza epidemics and are of the same size as meterologic effects. The results for the lower end of the dose range are in agreement with linear models fitted in studies of moderate air pollution and episode studies.

Objective: This paper represents the results of analyses of total daily mortality and air pollution data collected in Erfurt, Thuringen, in the former German Democratic Republic during the 1980s.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Median daily mean - 197µg/m^3 Daily mean range: 10-3569µg/m^3 Median daily max:420µg/m^3 Daily max range:10-4960µg/m^3	10 years	No info	No info	Roughly 217,000 Median total mortality (deaths/day) — 6	No info	n/a	n/a

Observations:

The best fit for pollution was obtained for log with a lag of 2 days. Daily mortality increased by 10% for an increase in SO_2 from 23 to 92 $\mu g/m^3$. The relative risk for the comparison of the log daily mean SO_2, 95% quantile versus the 5% quantile ($929\mu g/m^3$ versus $23\mu g/m^3$), lag 2 days, was calculated to be 1.10. This RR was significant with $p=0.01$.

For days with $SO_2 \geq 3000\mu g/m^3$ (only 4 days) , we predict 73% more deaths than expected under the logarithmic model, nearly 100% more than on days with very low pollution (5th quantile=$23\mu g/m^3$).

An analysis of the data by month, October (RR=1.09) and February through April (1.10-1.08) had the highest RRs, but the difference between months was not significant.

To check the consistency of results over years, the regression was refitted with SO_2 – years interactions. The results were fairly consistent. The difference between the year with the strongest effect (1987 RR=1.20) and the weakest (1988 RR = 1.03) is just close to being significant ($p=0.06$). There was no systematic change of the relationship over the years.

There was a small harvesting effect, but the results were not significant. High mortality about 2 weeks before an air pollution episode would lead to a smaller effect. The effect of the difference between the 95% quantile and 5% quantile of SO_2, which increases mortality on average by 10% and can be modified from 8% to 12%, comparing days with a high mortality in the previous 15 days. Therefore, the harvesting effect may modify the pollution effect by ±2%.

The effect for particulates was stronger than the effect of SO_2.

SO_2 has a very distinct seasonal pattern with a relatively sharp rise in values with the beginning of the heating season. The SO_2 series was highly autocorrelated; $r=0.85$ for the daily means and $r=0.77$ for daily maxima (1 day lag). The limited particulate data showed somewhat less autocorrelation ($r=0.60$). The same day correlation of particulates with daily mean SO_2 was not very strong ($r=0.55$), but the relationship appeared to be linear. A two-pollutant model and fair comparison of SO_2 and SP could only be calculated for 1988-89. The SO_2 effect alone is much smaller than for the whole period. In a two-pollutant model, the parameter for SO_2 is reduced to close to zero, while the particulate parameter decreases slightly. In earlier years, SO_2 does seem to act as a proxy for particulates.

*Statistically significant

Author's Conclusions:

"The effects of air pollution are smaller than the effects of influenza epidemics and are of the same size as meterologic effects. The results for the lower end of the dose range are in agreement with linear models fitted in studies of moderate air pollution and episode studies."

"The lack of overdispersion and autocorrelation in the residuals of the final model indicate a good fit of the model. After fitting SO_2, several variations and refinements were checked. Remodelling without meteorology made little difference in the pollution parameters. Meteorology may be an important covariate, but it is not an important confounder. Correcting only for annual variation slightly increased the SO_2 effect, so the parameter given here is somewhat conservative."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate

	for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **fixed monitors** Was the exposure concentration measured? (+) **10 yrs** Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? **(n/a)** Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel?

	(+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (-+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The effects of SO_2 on mortality were determined to be smaller than the effects of influenza epidemics and are of the same size as meterologic effects. The magnitude of the effect was small, 10%, when comparing the 95% quantile (929μg/m^3) to the 5% quantile (23μg/m^3). The SO_2levels likely do not accurately represent personal exposure.

However, the lack of overdispersion and autocorrelation in the residuals of the final model indicate a good fit of the model.

The results for the lower end of the dose range are in agreement with linear models fitted in studies of moderate air pollution and episode studies. Results show that a change in exposure concentrations does not lead to a change in mortality caused by SO_2.

Confidence index:

Only one monitor was used to measure SO_2 located inside a closed courtyard of five story buildings at about the fourth floor level. Comparisons were made with another station, but only for 3 of the 10 years.

Very detailed confounder analysis.

There is no detail on how missing data were accounted for.

The confidence intervals for the relative risks were not reported.

This study attempted to correlate episodes of high SO_2 concentrations in Erfurt with trends in mortality. The author clearly describes the monitoring process and identifies the location of the sampler (4th floor levels) as a potential confounder. However, the author correlates these findings with those out of Weimar to ensure that the SO_2 concentration at the 4th floor monitor is comparable to that breathed in. While the study assumes that concentration measured at the filter are comparable to that concentration breathed in by the population, it does not consider the distance that some subjects may live from the emission sources. The study utilizes a large population and, consequently, does not adequately describe group characteristics, possibly leading to biases in the findings.

The study design and conduct of the experiments were good in the report. Data analysis was complete and thorough.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Xu, X., Gao, J., Dockery, D.W., Chen, Y.	**Internal ID:** 338

Title: Air Pollution and Daily Mortality in Residential Areas of Beijing, China

Year: 1994

Abstract: The relationship between air pollution and daily mortality in 1989 was examined in two residential areas in Beijing, China. Very high concentrations of sulfur dioxide (SO_2) (mean = 102 micrograms/m^3, maximum = 630 micrograms/m^3) and total suspended particulates (TSPs) (mean = 375 micrograms/m^3, maximum = 1,003 micrograms/m^3) were observed in these areas. Daily counts of deaths were regressed, using Poisson regression on the logarithm of (SO_2) and/or TSPs controlling for effects of temperature, humidity, and day of week. A highly significant association was found between ln (SO_2) and daily mortality. The risk of total mortality was estimated to increase by 11% (95% confidence interval [95% CI] = 5%-16%) with each doubling in SO_2 concentration. Separately, the association of ln(TSP) with total daily mortality was positive but not significant (4% increase in mortality with each doubling in TSP; 95% CI = -2%-11%). When mortality was analyzed separately by cause, the association with a doubling in SO_2 was significant for chronic obstructive pulmonary disease (29%), pulmonary heart disease (19%), and cardiovascular disease (11%), and marginally significant for the other nonmalignant causes (8%), but not statistically significant for cancer (2%). A similar association was noted for a doubling in TSP (4%, 38%, and 8% for total, chronic obstructive pulmonary disease, and pulmonary heart disease mortality, respectively), but the result was only statistically significant for chronic obstructive pulmonary disease. In the season-specific analysis, both SO_2 and TSP were found to be significant predictors of total daily mortality in summer. In winter, SO_2 was again significantly associated with increased mortality, but no positive association was found between TSP and mortality. In the cause-specific analysis, the strongest effects of SO_2 and TSP on mortality were consistently seen for respiratory diseases in both seasons. This study shows increased mortality associated with air pollution at SO_2pollution levels below the current WHO recommendations.

Objective: Data from two residential areas in Beijing with very high ambient air pollution concentrations of particulates and sulfur dioxides from domestic coal burning provide an opportunity to evaluate the association between daily mortality and air pollution in a setting very different from that considered in the United States studies.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean 102.3μg/m^3 Maximum:630μg/m^3	334 days	Male and female	All	1 419 123 subjects; Mean deaths per day: 21	No info	n/a	n/a

Observations:

When ln(SO_2) was included in the model separately, ln(SO_2) on the same day was a highly significant predictor of daily mortality.
The risk of all cause mortality was estimated to increase by 11% (95% CI 5-16%) with a doubling in SO_2. When mortality was analyzed separately by cause of death, the association with a doubling in SO_2 was significant for chronic obstructive pulmonary diseases (29% 95% CI=4-53%); pulmonary heart disease (19,

95% CI=6-31%); and cardiovascular disease (11%, 95% CI=4-18%); and marginally significant for the other nonmalignant causes, but not statistically significant for cancer. A similar association was noted for a doubling in TSP (4%, 38% and 8% for total, chronic obstructive pulmonary disease, and pulmonary heart disease mortality, respectively), but the result was only statistically significant for chronic obstructive pulmonary disease.

SO_2 and TSP concentrations were much lower in the summer season than in the winter season. When the analysis was performed separately by season, SO_2 was found to be a significant predictor of total daily mortality in summer. The association remained significant when both pollutants were included simultaneously in the model. In winter, SO_2 was again associated significantly with increase mortality, but no positive association was found between TSP and mortality. In the cause-specific analysis, the strongest effects of SO_2 and TSP on mortality were consistently seen for respiratory diseases in both seasons. The association of chronic obstructive pulmonary diseases was associated significantly with SO_2 in winter and with TSP in summer. A significant relationship between pulmonary heart disease and SO_2 was found in both seasons.

*Statistically significant

Author's Conclusions:

This study shows increased mortality associated with air pollution at SO_2 pollution levels below the current WHO recommendations.

"If SO_2 increases daily mortality, it must affect the respiratory system first, i.e., persons with serious respiratory illness should be expected to have higher risks. The results from these analyses are consistent with this hypothesis. The increased risk for pulmonary heart disease and chronic obstructive pulmonary disease mortality associated with SO_2 were, respectively, 1.7 and 2.6 times higher than for all-cause mortality. In contrast there was no relative increase in cardiovascular disease mortality, compared with all-cause mortality, and no effect was found for cancer mortality."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate?

	(+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (n/a no symptom data) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? no extrapolations made Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

A highly significant association was found between $\ln(SO_2)$ and daily mortality at levels of SO_2 below the WHO guideline. The increased risks for pulmonary heart disease and chronic obstructive pulmonary disease mortality may indicate that persons with serious respiratory illness have higher risk.

While monitoring at the source of the emission assumes that the population lives nearby and is exposed to this concentration, in this study the population is unique in that a large number of subjects reside within 10 km of the two ambient air pollution monitors.

The association of mortality with SO_2 in Beijing was significant in both summer and winter. Most studies done in other regions have found the association to be more significant in winter. Ambient air monitoring data in winter may reflect an individual's actual exposure less accurately than in summer because of reduced ventilation of indoor spaces and indoor sources such as coal burning.

Confidence index:

The introduction was very brief.
There was only one monitor per residential area.
SO_2 data was only available 2-3 weeks of every month. There were only 194 d of pollution data available for this analysis. Selection of days to collect air samples was randomly done.
A large number of subjects resides within 10 km of one of the two monitors, which provides a large sample size.
Some of the confidence intervals were wide.
Indoor air pollution is not considered even though it may be a significant confounder. Coal stoves are used for heating and cooking.
Sensitivity of the monitoring devices was not mentioned.

Review – Confidence index ranking:

Moderate to low

CASE-CONTROL REVIEW FORM

Author: Walters, S., Griffiths, R.K., Ayres, J.G.	**Internal ID:** 340

Title: Temporal association between hospital admissions for asthma in Birmingham and ambient levels of sulphur dioxide and smoke

Year:1994

Abstract: BACKGROUND--A study was performed to determine whether daily and weekly variations in the levels of smoke and sulphur dioxide (SO_2) in Birmingham are related to hospital admissions for asthma and acute respiratory diseases. METHODS--Daily numbers of hospital admissions for asthma (ICD code 493) and acute respiratory conditions (ICD 466, 480-486, 490-496) for residents of Birmingham between 1988 and 1990 were obtained from West Midlands RHA Korner inpatient data. Average daily levels of sulphur dioxide and smoke were obtained from Birmingham City Council for the same period, together with daily meteorological summaries from the Department of Geography, University of Birmingham. With the exception of one day, all air pollution measurements remained within current EC guide levels. Data were divided into seasons and the relation between hospital admissions and pollutant levels were explored by stepwise least squares regression models. Meteorological variables (temperature, pressure, humidity) were entered into the model if they showed significant association with hospital admissions during the season in question. Analysis was undertaken for daily (same day and lagged by two days) and weekly pollutant levels. Admissions were lagged behind pollution levels to allow for delayed effects of pollutants. RESULTS--The mean daily level of smoke was 12.7 micrograms/m^3 and of SO_2 was 39.1 micrograms/m^3, with maxima of 188.3 micrograms/m^3 and 126.3 micrograms/m^3, respectively. Significant associations were found between hospital admissions for respiratory disease lagged by two days, and smoke and SO_2 levels during winter. Associations between admissions for asthma and smoke and SO_2 levels were significant at the 5% level. These were independent of temperature, pressure, and humidity. Stepwise regression including both pollutants showed that smoke, but not SO_2, was a significant independent predictor of hospital admissions for both asthma and all respiratory conditions. During winter a rise of 100 micrograms/m^3 smoke might result in five (95% CI 0.6 to 9) more asthma admissions and 21.5 (95% CI 10 to 33) more acute respiratory admissions each day in Birmingham. A 100 micrograms/m^3 rise in SO_2 might result in four (0 to 7) more asthma admissions and 15.5 (6 to 25) more respiratory admissions each day. Independent associations were also found between weekly mean smoke and SO_2 levels and all respiratory admissions during autumn and winter. During summer, daily mean smoke and SO_2 levels were significantly associated with non-lagged daily admissions for all respiratory diseases ($p < 0.02$). There was no association between air pollution and hospital admissions during spring. CONCLUSIONS--Daily variations in smoke and SO_2 levels are significantly associated with hospital admissions for asthma and respiratory disease during winter in Birmingham at levels of air pollutants within the EC guide levels. This association was independent of potential confounding effects of weather (temperature, pressure, humidity) and suggests that current levels of air pollution can still produce significant health effects.

Objective: This study sought to determine whether SO_2 and smoke were associated with hospital admissions for asthma and respiratory disease in Birmingham, to determine the size and potential public health importance of any association seen, and to establish whether these health effect could be detected using routinely collected data.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean: Spring- 42.87±1.35μg/m^3	April 1988-March 1990	Male and female	No info	Residents of Birmingham admitted to hospital from	No info	n/a	n/a

Summer- 37.77±1.17µg/m^3 Autumn- 40.91±1.24µg/m^3 Winter- 34.18±1.03µg/m^3 All year- 39.06±0.62µg/m^3				1988-1990			

Observations:

Relation between hospital admissions and air pollution

The mean daily level of smoke was 12.7 µg/m^3 and of SO2 was 39.1 µg/m^3 with maxima of 188.3 µg/m^3 and 126.3 µg/m^3 respectively.

Daily pollution levels and hospital admissions

During winter daily levels of SO$_2$were significantly associated with hospital admissions for all respiratory conditions at the 1% level. The association was seen for hospital admissions without and with a two day lag. The association between SO$_2$ and asthma fell just below the 5% significance level.

Stepwise regression models revealed SO$_2$ to be a significant independent determinant of hospital admissions for both asthma and all respiratory conditions at or below the 5% level.

Stepwise multiple regression including both pollutants revealed SO$_2$ not to be a significant independent determinant of wintertime hospital admissions for asthma and all respiratory conditions.

During summer, daily levels of SO$_2$ were significantly related to daily unlagged admissions for all respiratory diseases at the 2% level.

During winter a 100µg/m^3 rise in SO$_2$ might result in four (0 to 7) more asthma admissions and 15.5 (6 to 25) more respiratory admissions a day.

During winter a rise of 100 µg/m^3 smoke might result in five more asthma admissions and 21.5 more acute respiratory admissions each day in Birmingham.

Independent associations were also found between weekly mean smoke and SO$_2$ levels and all respiratory admissions during autumn and winter

Weekly pollution levels and hospital admissions

Significant associations were shown between all respiratory admissions and SO$_2$ levels in winter and between all respiratory admissions and SO$_2$ levels only during autumn.

Stepwise multiple regression models showed that during winter, weekly SO$_2$ levels were significantly associated with weekly hospital admissions for asthma ($p<0.005$). SO$_2$was not significant associated with SO$_2$. During autumn SO$_2$was significantly associated with weekly admissions for all respiratory diseases ($p<0.001$).

Author's Conclusions:

"Daily variations in smoke and SO$_2$ levels are significantly associated with hospital admissions for asthma and respiratory disease during winter in Birmingham at levels of air pollutants within the EC guide levels. This association was independent of potential confounding effects of weather (temperature, pressure, humidity) and suggests that current levels of air pollution can still produce significant health effects."

"We have shown that there is a significant association between hospital admissions for asthma and all respiratory conditions and air pollution levels during the winter at all levels of air pollution which remained within EC guide levels, with the exception of one day. Associations at other times of year were present but weaker. These associations were independent of significant confounding meteorological variables. Smoke, and not SO$_2$, was the important independent predictor of daily hospital admissions during winter, and of weekly admissions for all respiratory conditions. This confirms the potential importance of health effects of particulates from the studies of Pope et al. in Utah where PM_{10} was found to be a significant predictor of a wide range of respiratory morbidity at very low concurrent levels of SO_2. SO_2 was a significant predictor of weekly asthma admissions."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+/-) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-some) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?

G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (no extrapolations made Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Weak but significant associations were found between hospital admissions for respiratory disease lagged by two days, and SO_2 levels during winter. Associations between admissions for asthma and SO_2 levels were significant at the 5% level. A $\mu g/m^3$ rise in SO_2 might result in four (0 to 7) more asthma admissions and 15.5 (6 to 25) more respiratory admissions each day. Independent associations were also found SO_2 levels and all respiratory admissions during autumn and winter. During summer, daily mean SO_2 levels were significantly associated with non-lagged daily admissions for all respiratory diseases ($p < 0.02$). There was no association between air pollution and hospital admissions during spring. These results suggest that low levels of SO_2 may still cause health effects.

This study was the first recent study of acute health effects of air pollutants in the UK on hospital admissions for respiratory diseases. This study produced significant findings linking hospital admissions for asthma and all respiratory conditions and air pollution levels during the winter. These findings may only be relevant to Europe in that climatic conditions in North America vary significantly and could effect respiratory health.

This study concludes that variations in smoke and SO_2 levels contribute to the rate of hospital admissions for asthma and respiratory diseases around winter time. The authors point out that this is happening at levels of air pollutants within the EC guide levels. This means that current levels of air pollution can still produce significant health effects.

Confidence index:

Possible misdiagnosis and misclassification with hospital admission data.

There were 7 monitors measuring SO_2. However, locations and correlation were not reported.

There seemed to be statistical overkill: in total 60 multiple regression models were constructed, and seven additional bivariate regression were carried out to assess the degree to which hospital admission lagged behind pollutant levels.

Raw data is included.Perhaps the non significant results in the spring and summer are due to the lower number of admissions.

Due to the number of subjects used in this study, it was difficult to list subject characteristics and to list individual data. This study did not make clear the specific concentrations at which respiratory health was affected. Furthermore, the study was restricted by data availability regarding the effects of SO_2. Future more specific population-based studies should attempt to establish the weight of effect of environmental factors on respiratory health.

Objective, results, and extrapolations and interpretations were stated very clearly and well supported. Monitoring devices were not mentioned in the report.

Review – Confidence index ranking:

Moderate to low

CASE-CONTROL REVIEW FORM

Author: Emerson, P.A.	**Internal ID:** 342
Title:Air Pollution, Atmospheric Conditions and Chronic Airways Obstruction	
Year:1973	
Abstract: 32 subjects were studied weekly for up to 82 weeks to assess the relationship between respiratory function in patients suffering from chronic airways obstruction and changes in atmospheric conditions and air pollution. Five day averages were taken of atmospheric conditions and pollution before and after each patient visit, and these averages were compared with the pulmonary function measurements made at the corresponding attendances and the correlation coefficients calculated on the serial results.	
Objective: The present study was undertaken to assess the relationships between respiratory function in patients suffering from chronic airways obstruction and changes in atmospheric conditions and air pollution.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean: $193 \pm 105 \mu g/m^3$ Range: 38.5-$722 \mu g/m^3$	1 year	14 male 4 female	No info	18	Chronic airways obstruction	n/a	n/a

Observations:

Observations are reported for each individual. SO_2 was only significantly correlated with FEV_1 in one patient.
$SO_2$was only significantly correlated with MEFR in two subjects.
There was a significant correlation between FEV_1 and MEFR in 15 out of 18 patients.
The largest number of significant correlations was with temperature. Other significant correlations with FEV were found with humidity change in four patients; with atmospheric pressure in three patients and with wind speed in four patients.

*Statistically significant

Author's Conclusions:

"It is concluded that the amounts of SO_2 and smoke in the air in London have now fallen to levels which have little direct effect on pulmonary function in patients with chronic airways obstruction."

"It appears that the present levels of these pollutants in the atmosphere of London have now been reduced to such an extent that they seldom have any significant effect on the degree of airways obstruction in patients with chronic bronchitis and asthma, at least as assessed by tests of forced expiration. There was, however, a significant correlation between a fall in temperature and a worsening of lung function in a third of the patients."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (more info needed) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) study period – 1 year Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (-) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (more info needed) Were assessment measures sufficiently sensitive? (n/a no symptom data) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (-) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (-) Did the investigator appropriately interpret the statistics?

	(-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (more info needed) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no significant findings relating SO_2 exposure and chronic airways obstruction.

The author notes that these results should be assessed on the understanding that all attempts to relate single variables to atmospheric conditions or pollution to a patient's condition are an oversimplification of the problem since different factors may act at different points on the respiratory tract and it is most unlikely that any act independently. It is likely that, since low concentrations appear to produce an undesirable effect when accompanied by particulate matter, other such dependencies may occur.

It was concluded in the study that the amounts of SO_2 and smoke in the air in London have fallen to levels which have little direct effect on pulmonary function in patients with chronic airways obstruction.

Confidence index:

The sample size is very small.
The subject's results are only compared with themselves.
Each subject is analyzed individually and 9 of the subjects are combined.
The methods section is lacking a great amount of detail.
The follow-up ranged from 12 – 82 wks per subject. The number of visits varied considerably between subjects.
There is no information on the SO_2 monitoring.
Only correlation analysis is performed.
The study only cites 5 references.
There are no details on the study population. The study is poorly designed and there is too little detail provided on the methodology and results.
Measurement of pollutants was not made at the subjects' residences, but at a monitoring station some distance away from their homes. While climatic information was given, demographic information was lacking.

The assessment did not seem to be too thorough and the procedure in this study was not well thought out and defined. Timing and frequency of this study was not well defined either. Patients attended sessions for different lengths of time and all missed some visits. The study design does not limit confounding variables.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Lebowitz, M.D., Toyama, T., McCarroll, J.	**Internal ID:** 343
Title:The Relationship between Air Pollution and Weather as Stimuli and Daily Mortality as Responses in Tokyo, Japan, with Comparisons with Other Cities	
Year:1973	
Abstract: A comparative, stimulus-response analysis of 3 yr of Tokyo daily mortality in relationship to air pollution and weather , has yielded significant relationships between abnormal environmental events (stimuli) and subsequent high (excess) mortality events (responses). The relationships between the magnitudes of these events and the nature of these events are similar to those found in New Your. One can therefore generalize relationships between mortality and air pollution weather over the many subsets of different environmental situations and mortality experiences.	
Objective: To determine the relationship between air pollution and mortality in Tokyo over a 3-year period.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Average: 5.2±2.2ppb Winter Average:5.7±1.3 ppb Summer Average: 4.4±0.9ppb	April 1966-March 1969	No info	No info	Average # of daily deaths: 113.6±16	No info	n/a	n/a

Observations:

In multiple regressions, all results were significant. However, the multiple regression coefficients were not very high, with a coefficient of only 0.18 for sulfur dioxide. Correlations of the raw data variables show high correlations between daily deaths and temperature, and lower correlations between deaths and either of the air pollutants. When the effect of season and day of the week are removed, by use of standard scores, the correlations between deaths and temperatures decreased, as did those between death and air pollutants. Correlation coefficients between the magnitudes of the high (excess) mortality events and their antecedent, associated abnormal environment events are very high in the winters (r=0.65), lower in the summers(r=0.48), and high for the total period (r=0.57). This is similar to the results in the studies of New York, Philadelphia, and Los Angeles. There is also a slight increase in the correlation coefficient when all stimuli and all responses are considered.
*Statistically significant

Author's Conclusions:

"Comparisons made between Tokyo and New York support the contention that air pollutants (such as SO_2) have a contribution to increased mortality independent of weather. As these studies have shown, there are many different environmental conditions and mortality experiences. Use of this stimulus-response method differentiates these various conditions as events in time. The method proves very useful in analyzing these situations."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (more info needed)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (n/a mortality data) Was the timing and length of follow-up appropriate? (n/a mortality data)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (more data needed) Were appropriate endpoints examined? (+) mortality Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) mortalityWere accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (n/a no symptom data) Was the nature and severity of symptoms graded against an explicitly defined scale? (no info) Did qualified personnel conduct the exams?

	(more info needed) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-) Are the statistical methods used appropriate? (-) Are confidence intervals reported? **(more detail needed)** Did the investigator appropriately interpret the statistics? **(unclear)** Are the observed trends statistically significant?
G. Interpretations:	(-) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (-) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (-) Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There are no clear findings on the relationship of $SO_2$and daily deaths in Tokyo, Japan.

Findings from this study are novel since previous epidemiological studies in either New York or LA involve a completely different seasonal pattern and climate. Consequently, comparisons made between Tokyo and New York support the contention that air pollutants have a contribution to increased mortality independent of weather. This study supports the use of stimulus-response method as a means of differentiating various climatic conditions as events in time.

The results from this study, compared with those found in New York are similar. That said, one can generalize relationships between mortality and air pollution-weather over the many subsets of different environmental situations and mortality experiences. This is true even though the seasons provide a different environmental picture compared to those in New York as another index for the environment in the winter was created, and although there was some change in correlations between magnitudes of stimuli and responses, this change was not statistically different.

Confidence index:

The methodology section is lacking significant detail.

There is no information on collection of mortality data except to say the data was collected from death certificates.

Relative humidity was not included as a possible confounder.

There is limited discussion on the correlation of SO_2 and daily deaths. The correlation is just mentioned in the body of the results section.

The data was analyzed using an ‘environmental index’ and a ‘green index’, however, these methods are very poorly explained and very confusing.

The methods discussed in the results section to analyze the data make no sense. The first step in the method is to examine the “concordance between days of

abnormal environment". Abnormal environment is not clearly defined. It is very unclear. SO_2 is not mentioned.
The correlation data is not presented in a table anywhere in the paper.
This study provided recorded factors such as temperature, and cause of mortality. However, other demographic data was unavailable. Multiple stepwise regression analysis was used to delineate the importance of specific variables and the direction of the relationships. This study utilized 6-month seasons to allow for a sufficient number of cases to be studied once missing data was removed.
Its conclusions are similar to other studies in that air pollutants have a contribution to increased mortality independent of weather. This paper does not discuss confidence intervals nor does it include raw and individual data.

Review – Confidence index ranking:

Low

DRAFT

CASE-CONTROL REVIEW FORM

Author: Sunyer, J., Castellsague, J., Saez, M., Tobias, A., Anto, J.M.	**Internal ID:** 345

Title: Air Pollution and Mortality in Barcelona

Year:1996

Abstract: STUDY OBJECTIVES: Studies conducted in Barcelona reported a short term relation between daily air pollutant values and emergency department admissions for exacerbation of chronic obstructive pulmonary diseases and asthma. Air pollution in Barcelona is mainly generated by vehicle exhaust and is below the World Health Organization air quality guidelines. The acute relation between air pollution and mortality was assessed. DESIGN: Daily variations in total mortality, mortality in subjects older than 70 years, and cardiovascular and respiratory mortality were related with daily variations in air pollutants for the period 1985-91, using autoregressive Poisson regression models that allow to control for temperature, relative humidity and variables handling temporal and autoregressive patterns. MAIN RESULTS: Black smoke and sulphur dioxide (SO_2) were related to total mortality (relative risks (RR) for 100 micrograms/m^3 = 1.07 and 1.13 respectively), elderly mortality (RR = 1.06 and 1.13), cardiovascular mortality (RR = 1.09 and 1.14), and respiratory mortality (RR = 1.10 and 1.13); all the associations being statistically significant, except for respiratory mortality ($p = 0.07$). The association between SO_2 and respiratory mortality was stronger in summer (1.24, $p < 0.01$) than in winter (1.08, $p > 0.1$). Oxidant pollutants (nitrogen dioxide and ozone) were positively related with elderly mortality (RR = 1.05 and 1.09, respectively) and cardiovascular mortality (RR = 1.07 and 1.09) during the summer ($p < 0.05$), but not during the winter. CONCLUSIONS: Current air pollutant levels were related to mortality in Barcelona. These results were consistent with similar studies in other cities and coherent with previous studies with emergency room admissions in Barcelona.

Objective: Studies conducted in Barcelona reported a short-term relation between daily air pollutant values and emergency department admissions for exacerbation of chronic obstructive pulmonary diseases and asthma. Air pollution in Barcelona is mainly generated by vehicle exhaust and is below the World Health Organization air quality guidelines. The acute relation between air pollution and mortality was assessed.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
24 h average: Winter-46 (2.2-160) µg/m^3 Summer-36.4(5.4-116.5)	1985-1991	No info	All ages	Median total mortality: Winter-48/d Summer-43/d	No info	n/a	n/a

Observations:

The adjusted relative risks and 95% confidence intervals of mortality were calculated per each 100µg/m^3 increase in SO_2. For the whole year, SO_2 levels were significantly associated with total mortality (RR=1.127(1.068-1.189)), mortality in $\geq$70y (RR=1.135 (1.065-1.209)) and cardiovascular mortality (RR=1.145 (1.063-1.232)). For the winter SO_2 levels were significantly associated with the total mortality (1.150 (1.076-1.229)), mortality $\geq$70y (RR=1.172 (1.083-1.267)), and cardiovascular mortality (RR = 1.147 (1.047-1.255)). For the summer, SO_2 levels were associated with total mortality, mortality $\geq$70y, cardiovascular

mortality and respiratory mortality (RR=1.244 (1.039-1.490)). with similar RR values as above. Black smoke and SO_2 were related to total mortality, elderly mortality, and respiratory mortality; all associations being statistically significant, except for respiratory mortality. The association between SO_2 and respiratory mortality was stronger in summer than in winter. Oxidant pollutants were positively related with elderly mortality and cardiovascular mortality during the summer, but not the winter.
*Statistically significant

Author's Conclusions:

"Current air pollutant levels were related to mortality in Barcelona. These results were consistent with similar studies in other cities and coherent with previous studies with emergency room admissions in Barcelona."

"We found a significant association between BS and SO_2 and total and specific mortality for cardiovascular and respiratory diseases. The associations between SO_2 and mortality groups were stronger than those with particles, in particular the association with respiratory causes. These results agree with previous reports in Barcelona. The magnitude of the associations found for both pollutants was in the upper range of those reported."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	Was the exposure concentration measured? Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) mortality Were appropriate endpoints examined? (n/a mortality) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset

	and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Significant associations between SO_2 and total and specific mortality for cardiovascular and respiratory diseases were found. In the summer, the association between SO_2 and respiratory mortality was higher for the summer than the winter, but this difference was not significant.

Barcelona offered a useful environmental scenario to study air pollution health effects based on its mild climate. Furthermore, the main pollution in Barcelona is vehicle exhaust. The authors compared findings from the current study with those from a previous study on emergency admissions. The mortality data was also able to extend data series used for emergencies. The author notes that attempting to relate individual pollutants to health effects faces the impossible task of measuring complex exposures such as urban pollution. Finally, the author notes that the reproducibility of positive associations between air pollutants and mortality in different places differs with the lack of clear biological explanations for how a small increase in some pollutants may cause death.

This study shows that SO_2 and oxidants showed stronger relations with respiratory and cardiovascular mortality, respectively, during summer than winter. An explanation is unknown, but the authors conclude that summer probably offers a much better scenario than winter in which to study the health effects of air pollution in places like Barcelona.

Confidence index:

The standard methodology of the APHEA project was used for this study.

More detail is needed on the statistical analysis, particularly confounders.

There is information lacking on the monitoring systems and the methods section could use a little more detail.

The introduction is fairly brief with few references.

This study attempted to control the possible confounding effect related to meteorology and temporal effects. Of particular importance was the association and interaction that they might have with the pollutants. Mortality data was collected from a register with a good reputation for completeness of information.

Unfortunately, data was collected from only seven manual samplers, placed around the city. This may not have provided sufficient data from a large city such as Barcelona.

Interpretations and conclusions are well explained. With observations that do not lead to absolute conclusions, numerous possible explanations are given. The main conclusion, that SO_2 is related to mortality in Barcelona, is consistent with similar studies in other cities. Exposure levels were not given in this study.

Review – Confidence index ranking:

Moderate

CASE-CONTROL REVIEW FORM

Author: Ponce de Leon, A., Anderson, H.R., Bland, J.M., Strachan, D.P., Bower, J.	**Internal ID:** 346

Title: Effects of air pollution on daily hospital admissions for respiratory disease in London between 1987-1988 and 1991-1992

Year: 1996

Abstract: STUDY OBJECTIVE: To investigate whether air pollution levels in London have short term effects on hospital admissions for respiratory disease. DESIGN: Poisson regression analysis of daily counts of hospital admissions, adjusting for effects of trend, seasonal and other cyclical factors, day of the week, holidays, influenza epidemic, temperature, humidity, and autocorrelation. Pollution variables were particulates (black smoke: BS), sulphur dioxide (SO_2), ozone (O_3), and nitrogen dioxide (NO_2), lagged 0-3 days. SETTING AND PATIENTS: All immediate admissions for respiratory disease (ICD 460-519) to hospitals in London health districts in the five years April 1987 to February 1992 for all ages and the 0-14, 15-64, and 65+ age groups. MAIN RESULTS: O_3 (lagged one day) was significantly associated with an increase in daily admissions among all age groups, except the 0-14 group, and this effect was stronger in the "warm" season (April-September). In this season, the relative risks of admission associated with an increase in 8 hour O_3 levels of 29 ppb (10th to 90th centile) were 1.0483 (95% CI 1.0246, 1.0726), 1.0294 (0.9930,1.0672), 1.0751 (1.0354,1.1163), and 1.0616 (1.0243,1.1003) for all ages and age groups 0-14, 15-64, and 65+ respectively. Very few significant associations were observed with the other pollutants, though these tended to be positive. Controlling for other pollutants made little difference to the O3 coefficients. There was evidence of a threshold at about 40-60 ppb O3 (maximum hourly or maximum 8 hour). CONCLUSIONS: O_3 levels in London have a small but significant effect on hospital admissions for respiratory disease at all ages. The possible role of aerollergen as a confounding factor needs to be examined. Unlike other cities where similar effects have been reported, little or no effect of particulates was observed in London.

Objective: To investigate whether air pollution levels in London have short term effects on hospital admissions for respiratory disease

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Daily average: 32.2±12.6μg/m^3	Study period: April 1987-February 1992	No info	All ages. Separated into 3 groups: 0-14y 15-64y >64y	Average daily admissions all ages: 125.7±51.3	No info	n/a	n/a

Observations:

For SO_2, a weak relationship between admissions and all ages at levels greater than 60μg/m^3 was revealed.
The only observations reported as significant were for the 15-64y group (for an increase in SO_2 from the 10th (18μg/m^3) to 90th (49μg/m^3) percentile, during the cool season (RR=1.0389(1.0010-1.0783)) and for the 0-14y group (for a 10th -90th percentile increase in SO_2 18-43μg/m^3) during the warm season (RR=1.0468

(1.0066-1.0885). Both of these associations were for lag 1 day.
No significant associations were seen between SO_2 concentrations and hospital admissions.
For BS and SO_2, statistical significance was found in only three instances. BS lagged 1 day for children over the whole year (negative association), SO_2 lagged 1 day for children in the warm season (positive association, and SO_2 lagged 1 day for adults aged 15-64 in the cool season (positive association).

Author's Conclusions:

"While effects were observed with NO_2, SO_2, and BS in some age groups, these were not as consistent as or of similar size to those of O_3."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **fixed monitors**Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams?

	(+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (-) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(no specific SO2 conclusions) Do** the conclusions make biological sense? **(no extrapolations for SO_2)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings for SO_2 were inconsistent. The two observations reported as significant are described above. The RR were greater than 1; however, the 95% confidence intervals included RR=1.

For SO_2 statistical significance was only found in three instances, and findings were not as significant as those for O_3. This paper focuses mainly on the O_3 findings.

Confidence index:

Four stations were used for SO_2 monitoring and a significant correlation between stations was reported. However, these data were not shown.

The choice of subjects was not based on residence of the patients.

A standard method was used – APHEA approach for the study design.

The methods used to control for confounders were extensively described.

There was considerable data manipulation , i.e. several dummy variables, smoothing that may have affected the observed association.

The authors report RRs as being significant when the 95% CI includes the null. The authors use too many significant figures.

There are no explanations for the inconsistent SO_2 results.

This study recorded the average climatic conditions and indicated that the major sources of pollution were motor vehicles and industry in some areas of the city. This study also attempted to correct for deseasonalisation, detrending, and calendar corrections.

SO_2 information/results in this study were very limited due to lack of statistical significance. Conclusions were focused on O_3, and very few associations were observed with the other pollutants. Also, equipment used to obtain environmental data was not appropriately described, and no raw data were provided.

Review – Confidence index ranking:

Low

DRAFT

CASE-CONTROL REVIEW FORM

Author: Ponka, A., Virtanen, M.	**Internal ID:** 347

Title: Asthma and ambient air pollution in Helsinki

Year: 1996

Abstract: OBJECTIVE: To study whether ambient air levels of sulphur dioxide (SO_2), nitrogen dioxide (NO_2), total suspended particulates (TSP), and ozone (O_3) affect the number of hospital admissions for asthma. DESIGN: The associations between the daily number of admissions and air pollutants were analysed with Poisson regression, taking into account potential confounding factors by using the standardised protocol of the APHEA project. SETTING: Helsinki, Finland, 1987-89. PATIENTS AND MEASUREMENTS: Patients admitted to hospitals through emergency rooms because of asthma (n = 2421). The daily mean concentration was 13-25 micrograms/m^3 for SO_2, 33-41 micrograms/m^3 for NO_2, 19-41 micrograms/m^3 for O_3, and 58-109 micrograms/m^3 for TSP during the different seasons. Values are means for various stations. The daily mean temperature during the three year period was +5.4 degrees C (range -37-27 degrees C). MAIN RESULTS: Positive associations with admissions were observed for O_3 levels in all children under 14 years, and for SO_2 levels in 15-64 year olds and among those older than 64. Significant associations were also seen between admissions for digestive tract diseases (the control) and O_3 levels. This suggests that the modelling, which proved to be problematic, was unsatisfactory, or it may be a statistical coincidence. A rise in temperature was associated with a low number of admissions for asthma among 0-14 year olds and among 15-64 year olds, whereas humidity did not have a significant effect on the number of admissions. CONCLUSIONS: It is possible than even low level pollution may increase hospital admissions for asthma. However, definitive conclusions will be justified only after meta-analysis comprising several studies. The methodology was seen to have a strong effect on the results and standardised methods, possibly differing from those for the study concerning mortality, are needed to investigate the association between morbidity and ambient air pollutants.

Objective: To study whether ambient air levels of sulphur dioxide (SO_2), nitrogen dioxide (NO_2), total suspended particulates (TSP), and ozone (O_3) affect the number of hospital admissions for asthma.

Overall study design:

Exposure level	**Exposure frequency/ duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Means: December-February: 26µg/m^3 March-May: 22µg/m^3 June-August: 13µg/m^3 September-November: 15µg/m^3	1987-1989	No info	Three groups: 0-14y; 15-64y; >64y	Total number of admissions: 2421	No info	n/a	n/a

Observations:

Positive associations with admissions were observed 24 h for SO_2 levels in 15-64 year olds (p=0.046) and among those older than 64 (p=-0.012). Significant associations were also seen between admissions for digestive tract diseases (the control) and O_3 levels. This suggests that the modeling that proved to be problematic, was unsatistactory, or it may be a statistical coincidence. A rise in temperature was associated with a low number of admissions for asthma among 0-14 year olds and among 15-64 year olds, whereas humidity did not have a significant effect on the number of admissions.

Author's Conclusions:

It is possible than even low level pollution may increase hospital admissions for asthma. However, definitive conclusions will be justified only after meta-analysis comprising several studies. The methodology was seen to have a strong effect on the results and standardised methods, possibly differing from those for the study concerning mortality, are needed to investigate the association between morbidity and ambient air pollutants.

"The modeling was problematic. In the first attempts, over correction for cyclicity and under correction for cyclicity and under correction for autocorrelation were made…In the present study, not only average values of pollutants measured at different stations, but also the measurements at the station with the highest values were used first in the analysis. The results of these analyses were in part illogical, showing that these measurements do not sufficiently reflect the exposure of the population to pollutants. The mean daily SO_2 values measured in Helsinki were lower than the maximum value recommended by World Health Organization – 125 $\mu g/m^3$. The daily guideline was not exceeded. However, a significant association was seen between daily SO_2 concentrations and the daily counts of emergency room admissions among the 15-64 year old patients and among those over 64 years old. However, this association was not observed among children. The inconsistency of the findings may suggest that there is no real effect. The other possibility is that there is a relation between the number of patients admitted for asthma and the presence of SO_2 at lower concentrations than previously believed harmful or than are listed in the guidelines given in many countries and by the World Health Organization."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **((more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)

E. Data collection:	(n/a on controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(more info needed) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (-) Did the investigator appropriately interpret the statistics? (few) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (more info needed) Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.,* additional chemical exposure, etc.)? (no SO2 conclusions) Do the conclusions make biological sense? (no extrapolations) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings in this study were very inconsistent. The authors report that due to serious problems with modelling the data, definitive conclusions cannot be made from this study. However, the inconsistency of the findings may indicate that there is no real effect. The other possibility is that there is a relation between the number of patients admitted for asthma and the presence of SO_2 at lower concentrations than previously believed harmful or that are listed in the guidelines given in many countries and by the WHO.

Confidence index:

Standard methodology of the APHEA project was used. However this protocol was developed to study mortality, not morbidity.
Major difficulty with the statistical modeling prevents definitive conclusions from being made, as reported by the authors.
No information in the hospital records of the residence of the patient.
As reported by the authors, the measurement of SO_2 does not sufficiently reflect the exposure of the population to SO_2.
This study considered possible confounding factors such as season, epidemics, day of week, holidays and temperature and relative humidity. The range of SO2 concentrations studied along with exposure period was recorded, and the number of admissions for diseases of the digestive system was used as a control.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Spix, C., Wichmann, H.E.	**Internal ID:** 348

Title: Daily Mortality and air pollutants: findings from Koln, Germany

Year: 1996

Abstract: STUDY OBJECTIVE AND DESIGN: For the APHEA study, the short term effects of air pollutants on human health were investigated in a comparable way in various European cities. Daily mortality was used as one of the health effects indicators. This report aims to demonstrate the steps in epidemiological model building in this type of time series analysis aimed at detecting short term effects under a poisson distribution assumption and shows the tools for decision making. In addition, it assesses the impact of these steps on the pollution effect estimates. SETTING: Koln, Germany, is a city of one million inhabitants. It is densely populated with a warm, humid, unfavourable climate and a high traffic density. In previous studies, smog episodes were found to increase mortality and higher sulphur dioxide (SO_2) levels were connected with increases in the number of episodes of croup. PARTICIPANTS, MATERIALS AND METHODS: Daily total mortality was obtained for 1975-85. SO_2, total suspended particulates, and nitrogen dioxide (NO_2) data were available from two to five stations for the city area, and size fractionated PM_7 data from a neighbouring city. The main tools were time series plots of the raw data, predicted and residual data, the partial autocorrelation function and periodogram of the residuals, cross correlations of prefiltered series, plots of categorised influences, chi 2 statistics of influences and sensitivity analyses taking overdispersion and autocorrelation into account. RESULTS AND CONCLUSIONS: With regard to model building, it is concluded that seasonal and epidemic correction are the most important steps. The actual model chosen depends very much on the properties of the data set. For the pollution effect estimates, trend, season, and epidemic corrections are important to avoid overestimation of the effect, while an appropriate short term meterology influence correction model may actually prevent underestimation. When the model leaves very little over-dispersion and autocorrelation in the residuals, which indicates a good fit, correction for them has consequently little impact. Given the model, most of the range of SO_2 values (5th centile to 95th centile) led to a 3-4% increase in mortality (significant), particulates led to a 2% increase (borderline significant, less data than for SO_2), and NO_2 had no relationship with mortality (measurements possibly not representative of actual exposure). Effects were usually delayed by a day.

Objective: For the APHEA study, the short term effects of air pollutants on human health were investigated in a comparable way in various European cities. Daily mortality was used as one of the health effects indicators. This report aims to demonstrate the steps in epidemiological model building in this type of time series analysis aimed at detecting short term effects under a poisson distribution assumption and shows the tools for decision making. In addition, it assesses the impact of these steps on the pollution effect estimates.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Median of daily means: $44\mu g/m^3$ Median of daily max: $91\mu g/m^3$	1975-1985 (approximately 4018 days with data)	No info	No info	Median cases per day: 29	No info	N/a	n/a

Observations:

SO_2 showed a small but significant relationship with mortality.
SO_2 (daily means) predicted a 3% increase in daily mortality for the range 20-122µg/m^3 (10th – 90th percentile) and lag 1 day.
SO_2 (daily max) predicted a 3% increase in daily mortality for the range 42-231µg/m^3 (10th – 90th percentile) and lag 1 day.
For the inner 90% range of SO_2 (23-118µg/m^3 for daily mean) (51-209µg/m^3 for daily max) averaged over 4 days, SO_2predicted a 4% increase in mortality.

The SO_2 daily mean is correlated highly with PM_7 (0.68) and TSP (0.60). NO_2 is correlated with TSP (0.56) and some SO_2 (0.44). Correlations are on same day data, cross correlations did not show stronger correlations. Analysis by season showed somewhat stronger effects for all pollutants in summer. SO_2 in relation to particle categories gave no indication of a synergism. SO_2 effects were stronger when TSP was below 100 µg/m^3, but this was less consistent.

*Statistically significant

Author's Conclusions:

With regard to model building, it is concluded that seasonal and epidemic correction are the most important steps. The actual model chosen depends very much on the properties of the data set. For the pollution effect estimates, trend, season, and epidemic corrections are important to avoid overestimation of the effect, while an appropriate short-term meteorology influence correction model may actually prevent underestimation. When the model leaves very little overdispersion and autocorrelation in the residuals, which indicates a good fit, correction for them has consequently little impact.
"SO_2 and PM_7 had rather large coefficients when correcting only for trend. SO_2 showed the better fit when because of its stronger seasonal pattern, which picked up the seasonality in the mortality data…the autocorrelation we see comes from influencing processes such as season, epidemics, weather, and to a small extent air pollution, which are themselves strongly intrinsically positively autocorrelated."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitors Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval?

	(n/a no controls) Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) **(+)** Was the timing and length of follow-up appropriate? **(+)** Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? **(+)** Were appropriate endpoints examined? **(+) mortality** Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(+)** Were accepted assessment methods employed? **(+)** Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(+)** Did qualified personnel conduct the exams? **(+)** Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	**(+)** Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(more info needed)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	**(+)** Was the original objective addressed? **(-)** Are the results novel? **(+)** Was the design of the study appropriate to investigate the primary objective? **(+)** Were assumptions reasonable? **(+)** Are the methods reproducible? **(+)** Did either the study design or the statistical analysis limit confounding variables (*e.g.,* additional chemical exposure, etc.)? **(+)** Do the conclusions make biological sense? **(no extrapolations made)** Are the extrapolations reasonable? **(+)** Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The main focus of this study was to demonstrate the steps in epidemiological model building in time series analysis aimed at detecting short term effects under a poisson distribution assumption. The impacts of the various steps on the pollution effects estimates are also assessed. The authors determined that seasonal and epidemic correction were the most important steps. With regards to the associations between SO_2 and mortality, even though some of the relationships were significant or of borderline significance, the actual effect size was very small.
Most of the range of SO_2 values led to a 3-4% increase in mortality; so, mortality is associated with SO_2 and air pollutants. The paper concludes that, when modeling, the more parameters that are included, the less variance one will see. This study takes on more of a technical point of view, and does not give many conclusions on SO_2 associations with mortality.

Confidence index:

The standard method of the APHEA project was used.
Confidence intervals are not reported.
There is some detail on the monitoring stations such as the height of the detection inlet and the location of the monitors.
Correlation between the monitors was assessed.
Excellent detail on confounders and statistical model.
Some of the results presentations are a little unclear.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Touloumi, G., Samoli, E., Katsouyanni, k. | **Internal ID:** 349

Title: Daily mortality and “winter type” air pollution in Athens, Greece – a time series analysis within the APHEA project

Year:1996

Abstract: STUDY OBJECTIVE: There is evidence that air pollution in Athens between 1975 and 1987 had adverse short term health effects. The short term effects of "winter type" air pollution on the daily total number of deaths are investigated for the period 1987-91 as part of the European Community multi centre APHEA project. DESIGN: A temporal study using aggregated data is presented. The associations of the daily time series of three pollutants, sulphur dioxide (SO_2), black smoke (BS), and carbon monoxide (CO) and the daily total number of deaths in the Athens area were assessed. DATA AND METHODS: The average measurement from three stations was used for each pollutant. The daily number of deaths was recorded from the Athens Town Registry and the registries of the 18 municipalities contiguous to Athens. Data on the mean daily temperature (degree C) and relative humidity (%) were also used. Poisson autoregressive models that also allowed for overdispersion were used. Seasonality, other long term patterns, temperature, humidity, day of the week, and holidays were adjusted for. Several a priori defined pollutant transformations and lags were investigated. One day measurements as well as cumulative exposure effects were assessed. Effect modification by season as well as among pollutants was tested. MAIN RESULTS: Linear terms were used for all pollutants. The magnitude of the effect was greater at lags 0 for CO and 1 for BS and SO2 gradually declining after lag 1. For an increase of 100 micrograms/m^3 in SO_2 and BS there were corresponding increases (95% CI) of 12% (7%, 16%) and 5% (3%, 8%) in the daily total numbers of deaths, while for an increase of 10 micrograms/m^3 in CO the increase (95% CI) in the daily total number of deaths was 10% (5%, 15%). A significant interaction of the effects of SO_2 with season were found. The strongest effect was observed during the winter, when higher levels of SO_2 were observed. A stronger effect of SO_2 on the daily total number of deaths was observed when the levels of BS were > 100 micrograms/m^3. CONCLUSIONS: These results strengthen the evidence of a causal association between ambient particle, SO_2, or CO levels in the air and the daily total number of deaths and points to an important public health issue for the Athens population.

Objective: There is evidence that air pollution in Athens between 1975 and 1987 had adverse short term health effects. The short term effects of "winter type" air pollution on the daily total number of deaths are investigated for the period 1987-91 as part of the European Community multi centre APHEA project

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Average of 3 monitoring stations: Mean:$51.3\pm29.8\mu g/m^3$ Range: $6\text{-}361\mu g/m^3$ Mean levels at Monitoring stations: Pattison:$75\pm46\mu g/m^3$ Piracus:$54.6\pm37.3\mu g/m^3$	1987-1991 (1826 days)	No info	No info	Mean daily number of deaths:: 37.2 ± 8.0	No info	n/a	n/a

Smirni: $23.6\pm20.4\mu g/m^3$							

Observations:

For a $100\mu g/m^3$ increase in SO_2 the corresponding relative risk of mortality was 1.12 (1.07-1.16).
For an $87.2\mu g/m^3$ increase in $SO_2$the corresponding relative risk of mortality was 1.10 (1.07-1.13).
The strongest effect of SO_2 was observed during the winter when higher levels of SO_2 were observed. A stronger effect of SO_2 on the daily total number of deaths was observed when the levels of BS were >100 $\mu g/m^3$.
Linear terms were used for all pollutants. The magnitude of the effect was greater at lags 0 for CO and 1 for BS and SO_2 gradually declining after lag 1. For an increase of 100 ug/m^3 in SO_2 and BS there were corresponding increases (95% CI) of 12% (7%,16%) and 5% (3%,8%) in the daily total number of deaths.

Author's Conclusions:

"In the US studies, adverse effects have been primarily associated with the levels of particles (especially those with an aerodynamic diameter <10μ) and not SO_2 or CO, while in our study SO_2 seemed to be the most important pollutant…in conclusion, the analysis presented here strengthens the evidence of a causal association between ambient particle, SO_2, or CO levels in the air and the total daily number of deaths and points once more to an important public health issue in Athens."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	. (+) Is the number of subjects sufficient to produce statistically significant results? . (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? . (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? . (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? . (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	. (+) Was the exposure concentration measured? . (+) Is the exposure duration known?
C. Equipment:	. (+) Was a full description of the monitoring devices provided? . (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	. (+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? . (n/a no controls) Were appropriate controls recruited? . (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) . (+) Was the timing and length of follow-up appropriate? . (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)

E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? **(more information needed)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Statistically significant associations were observed between $SO_2$and daily mortality. However, the magnitude of these effects are relatively weak.

Confidence index:

The introduction is very brief with too few references.

Age and sex of the deceased not provided.
Standard methodology used – APHEA project.
The separate SO_2concentraitons for each monitoring station is provided.
There is no information given on the approximate locations of the monitoring systems within the city.
Confounding was addressed according to standard methodology.
Confidence intervals were reported using F-tests and Poisson autoregression was used.
The device for measuring air pollution was not described. Raw and individual data were not provided.

Review – Confidence index ranking:

Moderate to low

CASE-CONTROL REVIEW FORM

Author: Wojtyniak, B., Pickarski, T.	**Internal ID:** 350

Title: Short term effect of air pollution on mortality in Polish urban populations – what is different?

Year:1996

Abstract: STUDY OBJECTIVE: To assess, within the multinational European Community funded APHEA project, the relationship between daily ambient air pollution with sulphur dioxide (SO_2) and black smoke (BS) and the daily number of deaths from all causes except external, from cardiovascular diseases, and from respiratory diseases taking into account several possible confounding factors. DESIGN: Time series analysis with the application of Poisson regression to the counts of daily number of deaths from selected causes over several years. Data were obtained from the computer files of individual death records in four Polish cities. Criteria developed for all the centres participating in the project were applied to the process of model building. SETTING: Four Polish cities - Cracow (period 1977-89), Lodz (1977-90), Poznan (1983-90), and Wroclaw (1979-89). SUBJECTS: Permanent residents of the cities who died in a city of residence or in a surrounding region during the study period. MAIN RESULTS: There were significant positive associations between mortality from all causes excluding external ones and SO_2 and BS in Cracow and in Lodz and between mortality from cardiovascular diseases and SO_2 in Cracow alone. In other cities the association was either non-significant or significant but in the opposite direction. No significant positive association was found between respiratory deaths and these pollutants. CONCLUSIONS: Short term effect of air pollution on mortality in Polish urban population cannot be ignored. However, differences observed between the cities suggest that the association may be not so straightforward and therefore there is a need of further studies to identify additional factors that may modify and confound the association..

Objective: To assess, within the multinational European Community funded APHEA project, the relationship between daily ambient air pollution with sulphur dioxide (SO_2) and black smoke (BS) and the daily number of deaths from all causes except external, from cardiovascular diseases, and from respiratory diseases taking into account several possible confounding factors.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Medians: Cracow-74.1μg/m^3 Lodz-45.7μg/m^3 Poznan-41.0μg/m^3 Wroclaw-29.0μg/m^3	Study periods: Cracow-1977-1989 (4747 d) Lodz 1977-90 (5112 d) Poznan 1983-90 (2863 d) Wroclaw 1979-89 (4017 d)	No info	No info	Average # of deaths/day: Cracow-17 Lodz-27 Poznan-17 Wroclaw-13	No info	n/a	n/a

Observations:

A significant positive association of SO_2 and BS with mortality due to all causes excluding external causes was observed in Cracow and Lodz. Mortality from diseases of the circulatory system was also associated with the SO_2level, but only in Cracow. In other cities the association was either non-significant or significant but in the opposite direction. No significant positive association was found between respiratory deaths and these pollutants Some of the results are

surprising – the significant but negative association of deaths from all causes and cardiovascular causes with SO_2 in Wroclaw. In Wroclaw other lags in the models for these diseases were not significant. Significant relative risks associated with a twofold increase in the daily concentration of SO_2 levels were low, close to RR=1.
*Statistically significant

Author's Conclusions:

"Short-term effect of air pollution on mortality in Polish urban population cannot be ignored. However, differences observed between the cities suggest that the association may be not so straightforward and therefore there is a need of further studies to identify additional factors that may modify and confound the association."

"Three specific issues are important in our findings. Firstly, while in two cities, namely Cracow and Lodz, some negative effects of air pollution on mortality were observed, this was not true in the other two. Secondly, in none of the cities was a positive association between air pollution and mortality from respiratory diseases observed, while in some other studies this was found to be stronger than an association with general mortality. Thirdly, when the air pollution effect was observed it was greater for SO_2 than for BS, which contradicts the results from the US locations. However, in some European cities a significant association of SO_2 with mortality was observed. We do not suggest that the pollutants considered act independently since their levels are correlated and the correlation coefficients of mean daily concentrations ranged from 0.61 in Wroclaw to 0.81 in Lodz. However, possible synergy between pollutants, between pollutants and meteorological conditions, and between pollutants and season will be analyzed later, in the next stage of our study."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (+) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)

E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (n/a no symptoms) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (more analysis needed) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (no extrapolations made) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were significant positive associations between mortality from all causes excluding external ones and SO_2 in Cracow and in Lodz and between mortality from cardiovascular diseases and SO_2 in Cracow alone. In other cities the association was either non-significant or significant but in the opposite direction. No

significant positive association was found between respiratory deaths and SO_2. The results were inconsistent and this may suggest that the findings result from some patterns in the data that were not well dealt with by the core model and required a different method of modeling. There may be a discrepancy in the data quality. The populations in each city may differ, i.e. number of susceptible people.
The author concedes that findings from this study might be slightly confusing, particularly in light of recent studies. The author also questions the data quality, and wonders why there are significant differences in causes of death between the four cities. These concerns reduce the validity of the findings. Future studies should examine inherent socioeconomic differences between communities to explain relationships between air pollution and health effects.

Confidence index:
The method used to measure SO_2 differed between Cracow and the other 3 cities.
Table 1 does not give the units for SO_2 concentrations.
The Tables need more explanation of some of the variables.
Meteorological variables were only measured from one site in each city.
The results were inconsistent.
Age and sex of the subjects was not provided.
Poisson regression was used to estimate the relationship between air pollution and daily deaths. Potential confounding factors such as temperature and relative humidity were obtained from the Institute of Meteorology and Water Management and information on influenza cases was reported. In the study, the control group was identified as the cause of death being a disease of the digestive system.
The results from this APHEA study is similar to others in that the same conclusions are formed--the associations between mortality and SO_2 are significant. It also lacks the same information as the other studies: raw data, individual data, and monitoring equipment information.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: **Dab, W., Medina, S., Quenel, P., Le Moullec, Y., Le Tertre, A., Thelot, B., Monteil,C., Lameloise, P., Pirard, P., Momas, I., Ferry , R., Festy, B.**

Internal ID: 351

Title: Short term respiratory health effects of ambient air pollution: results of the APHEA project in Paris

Year:1996

Abstract: STUDY OBJECTIVE: To quantify the short term respiratory health effects of ambient air pollution in the Paris area. DESIGN: Time series analysis of daily pollution levels using Poisson regression. SETTING: Paris, 1987-92. MEASUREMENTS AND MAIN RESULTS: Air pollution was monitored by measurement of black smoke (BS) (15 monitoring stations), sulphur dioxide (SO_2), nitrogen dioxide (NO_2), particulate matter less than 13 microns in diameter (PM_{13}), and ozone (O_3) (4 stations). Daily mortality and general admissions to public hospitals due to respiratory causes were considered. The statistical analysis was based on a time series procedure using linear regression modelling followed by a Poisson regression. Meteorological variables, epidemics of influenza A and B, and strikes of medical staff were included in the models. The mean daily concentration of PM_{13} and daily 1 hour maximum of SO_2 significantly affected daily mortality from respiratory causes. An increase in the concentration of PM_{13} of 100 micrograms/m^3 above its 5th centile value increased the risk of respiratory death by 17%. PM_{13} and BS were also associated with hospital admissions due to all respiratory diseases (4.1% increased risk when the BS level exceeded its 5th centile value by 100 micrograms/m^3). SO_2 levels consistently influenced hospital admissions for all respiratory diseases, chronic obstructive pulmonary disease, and asthma. Asthma was also correlated with NO_2 levels. CONCLUSIONS: These results indicate that even though the relative risk is weak in areas with low levels of pollution, ambient air pollution, and especially particulate matter and SO_2, nonetheless require attention because of the number of people exposed and the existence of high risk groups.

Objective: To quantify the short term respiratory health effects of ambient air pollution in the Paris area.

Overall study design:

Exposure level	Exposure frequency/ duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
24hr: mean-29.7μg/m^3 median-23.0μg/m^3 5th centile-7.0μg/m^3 99th centile-125.0μg/m^3	1987-1992	No info	No info	Daily average deaths: 37 Mean number of daily hospital	varied	n/a	n/a

1h max: mean-59.9µg/m^3 median-46.7µg/m^3 5th centile-14.0µg/m^3 99th centile-232.7µg/m^3				admissions: Respiratory-79 Asthma-14 COPD 12			

Observations:

Relative risks are calculated for a concentration 100µg/m^3 greater than: the 24 hour reference value (calculated as the 5th centile of the concentration distribution) (see exposure level)

The daily count of deaths from respiratory causes was significantly associated with the mean daily 1 hour maximum of SO_2.

The relative risk associated with SO_2 (24hr) was 1.085 (1.015-1.159) for mortality. No other significant relations with mortality were observed.

24-hour concentrations of SO_2 significantly increased the relative risk of hospital admissions for respiratory diseases. The relative risk of admission for respiratory diseases and SO_2 (24hr) was 1.042 (1.005-1.080). The relative risk associated with SO_2 was less than half that of PM_{13}.

The relative risk of admission for COPD and SO_2 (24hr) was1.099(1.023-1.180) and for SO_2(1 hr max) was 1.051(1.025-1.077). These were significant for current day exposure.

The relative risk of admission for asthma and SO_2 (24hr) was 1.070(1.004-1.141).

The concentration of the preceding day was the best predictor, and the best fit was again obtained by a log transformation of the pollutant series. Although the relative risks were approximately the same for the 24h mean concentrations of SO_2 and BS, they fell short of statistical significance. SO_2 indicators were associated with a significant elevated risk with no lag.

*Statistically significant

Author's Conclusions:

"These results indicate that even though the relative risk is weak in areas with low levels of pollution, ambient air pollution, and especially particulate matter and SO_2, nonetheless require attention because of the number of people exposed and the existence of high risk groups."

"Air pollution in the Paris region does indeed have adverse effects on short term respiratory health, as indicated by the weak but consistent relative risks that are characteristic of low pollution areas. The number of persons exposed and the presence of especially vulnerable groups such as those with asthma point to the need for increased attention to these results. Although the risks observed in the general population are weak, it is reasonable to assume that they are much higher among susceptible groups. Our findings for asthma provide support for this hypothesis."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made?

	(n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable?

	(+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The relative risks were small, but may still be significant given the size of the population. However, some of the relative risks reported as significant included RR=1 in the confidence interval.

This study determined that ambient air pollution has a short-term adverse health effect. The author notes two strengths of the study were the size of the population and the use of the influenza monitoring system. However, its limitations may be the inability to distinguish emergency from planned hospital admissions and the quality of PM_{13} measurements.

This study indicates that even though relative risk is weak in areas with low levels of pollution, the need is still there to attend to the pollution levels because of the number of people exposed and because of the high risk groups.

Confidence index:

Standard methodology used for data collection and assessment – APHEA protocol.

Four stations measured SO_2 and were calibrated every 2 weeks.

Admissions data was available from 27 hospitals (approximately 800,000 emergency and planned admissions per year).

Emergency admissions cannot be distinguished from planned admissions.

Good detail provided regarding accounting for missing SO_2 data.

The location of the monitors is not provided.

This study considered meteorological variables as confounders and included them in the modeling process. In addition, the study used an autoregressive Poisson model and took into account the effects of a strike of medical workers since this affected level of hospital admissions.

The quality of the study design, conduct and reporting is consistent with the other APHEA studies except that in this study, confidence intervals have not been recorded.

Review – Confidence index ranking:

Moderate to low

CASE-CONTROL REVIEW FORM

Author: Zmirou, D., Barumandzadeh, T., Balducci, F., Ritter, P., Laham, G., Ghilardi, J.P.

Internal ID: 352

Title: Short-term effects of air pollution on mortality in the city of Lyon, France, 1985-1990

Year:1996

Abstract: OBJECTIVE: The short term association between daily mortality and ambient air pollution in the city of Lyon, France (population, 410,000) between 1985 and 1990 was assessed using time series analysis. DESIGN: This study followed the standardised design and statistical analysis (Poisson regression) that characterize the APHEA project. METHODS: Four categories of cause of death were studied: total (minus external causes), respiratory, cardiovascular, and digestive causes (as a control condition). RESULTS: No association was found with any cause of death for nitrogen dioxide (NO_2) and ozone (O_3), nor, for any pollutant, for digestive conditions. Sulphur dioxide (SO_2) and, to a much lesser degree, suspended particles (PM_{13}), were significantly related to mortality from respiratory and cardiovascular conditions. The relative risk (RR) of respiratory deaths associated with a 50 micrograms/m^3 increment of mean daily SO_2 over the whole period was 1.22 (95% CI 1.05, 1.40); the RR for cardiovascular deaths was 1.54 (1.22, 1.96). The corresponding RRs for PM_{13} were 1.04 (1.00, 1.09) for respiratory mortality and 1.04 (0.99, 1.10) for cardiovascular deaths. CONCLUSIONS: The effects of particulates were slightly increased during the cold season. When particulates concentrations were greater than 60 micrograms/m^3, the joint SO_2 effect was increased, suggesting some interaction between the two pollution indicators. These results agree with other studies showing an association between particulate pollution and daily mortality; however, they also suggest the noxious effect of SO_2.

Objective: The short term association between daily mortality and ambient air pollution in the city of Lyon, France (population, 410,000) between 1985 and 1990 was assessed using time series analysis.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
SO_2Mean: Mean-46.76µg/m^3 Range-2.16-314.57µg/m^3 SO_2 max: Mean-100.22µg/m^3 Range-4.71-635.69µg/m^3	1985-1990	No info	No info	Mean total deaths per day:6.43	No info	n/a	n/a

Observations:

The relative risks of mortality in relation to cause that were associated with a 50μg/m^3 increase in the level of pollutants over the whole 1985-1990 period were calculated. Since only relatively "moderate to low" levels of pollution were observed during the study period, this computation is most valid in the range of 0-150μg/m^3. Typically, the RRs represent the relative increase in mortality counts when the levels of pollutants step from 25 to 75μg/m^3 (or 50 to 100μg/m^3).

SO_2showed a clear association with mortality in the city of Lyon; it was associated with an increase in all causes of mortality except digestive conditions over the whole study period (control).

Relative Risks

	SO_2 Mean	Max
Total minus external causes	1.06(1.02-1.09) lag0	1.29 (1.07-1.55) lag0
Respiratory deaths	1.05(1.02-1.09) lag3	1.02 (1.01-1.03) lag0-3
Cardiovascular deaths	1.08(1.03-1.12) lag0-3	1.03(1.01-1.05)lag 1

SO_2 is the only pollutant that shows a clear association with mortality in the city of Lyon; it is associated with an increase in all causes of mortality except digestive conditions over the whole study period. No effect of particles was apparent over the whole study period for total mortality, but this is of borderline significance for respiratory and cardiovascular deaths.

Author's Conclusions:

"The effects of particulates were slightly increased during the cold season. When particulates concentrations were greater than 60 micrograms/m^3, the joint SO_2 effect was increased, suggesting some interaction between the two pollution indicators. These results agree with other studies showing an association between particulate pollution and daily mortality; however, they also suggest the noxious effect of SO_2."

"This study along with many other APHEA data described in this supplement add to the findings of previous short term mortality studies on particulate air pollution. It also stresses some characteristic of western European urban air where SO_2 pollution still plays an important noxious role."

"The main results of this study are the following: (1) only SO_2 and suspended particles showed an association with index categories of mortality; (2) SO_2 was significantly associated with an excess risk of total deaths and deaths from respiratory and cardiovascular conditions. The effect on respiratory mortality was increased when particulate concentrations were high; no seasonal modification or interaction with particulate pollution was noted for cardiovascular mortality; the regression coefficients were weaker than for SO_2.

People who have died on 'high' pollution days were on average a little older than those who died on other days. When SO_2 concentrations exceeded 100 μg/m^3, the proportion of people older than 70 among those who died was greater. This result supports other findings showing that the elderly and those with acute or chronic respiratory and cardiac conditions are the sensitive population. SO_2 and particulates per se may not be the primary aggressors of the respiratory tract, but they can exacerbate pre-existing conditions such as asthma, chronic bronchitis and emphysema."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (+) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (+) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (n/a no symptom data) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (some) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics?

	(+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

$SO_2$was significantly associated with an excess risk of total deaths (minus external causes) and deaths from respiratory and cardiovascular conditions. As reported by the authors, Lyon is the smallest town in the APHEA project, and this affects the counts of daily mortality(0.1%, 65%, 14% and 68% of days throughout the whole period had no mortality events, for total deaths minus external causes an deaths for respiratory , cardiovascular, and digestive conditions, respectively). This size limitation hampers a clear conclusion, on the subtle season or pollutants' combination effects.

While a previous study on the same theme had been done in 1974-76, physico-chemical nature of particulates had likely changed over the last decade.

This study adds to the findings of previous short term mortality studies on particulate air pollution. It also stresses some characteristics of western European urban air. There are indications from this study that there is some interaction between two pollution indicators.

Confidence index:

The relative risks reported in the abstract don't match what is reported in Table 4.

Standard methodology followed – APHEA project.

The authors gave reasons for choosing the monitoring sites.

Details on estimation of missing data were provided.

Deaths due to digestive diseases were used as control.

Good detail was provided on the statistical analysis.

Excellent detail on descriptive and confounding variables included in the final models.

Strict rules were set for the assessment of population exposure in order to facilitate comparisons with APHEA cities. While data was monitored at multiple stations, because of instrument failure the average SO_2 measured was used.

Review – Confidence index ranking:

Moderate to high

CASE-CONTROL REVIEW FORM

Author: **Schouten, J.P., Vonk, J.M., de Graaf, A.**

Internal ID: 353

Title: **Short-term effects of air pollution on emergency hospital admissions for respiratory disease: results of the APHEA project in two major cities in The Netherlands, 1977-89**

Year: **1996**

Abstract: OBJECTIVE: To assess the short term relationship between air pollution and the daily number of emergency hospital admissions for respiratory disease. DESIGN: Data were analysed using autoregressive Poisson regression allowing for overdispersion and controlling for possible confounding factors such as seasonal and other chronological variables, meteorological factors, and influenza epidemics. SETTING: The two major cities in The Netherlands-Amsterdam (694,700 inhabitants) and Rotterdam (576,200 inhabitants). PARTICIPANTS AND MEASUREMENTS: Emergency hospital admissions for respiratory diseases, registered on a daily basis by the National Medical Registration, for the period 1977-89 were used. ICD-9 codes included were: respiratory (460-519), chronic obstructive pulmonary disease (490-492, 494, 496), and asthma (493). The mean (range) of the total daily number of admissions for these three classifications were as follows: 6.70 (0-23), 1.74 (0-9) and 1.13 (0-7) respectively in Amsterdam and 4.79 (0-19), 1.57 (0-9), and 0.53 (0-5) in Rotterdam. Air pollution measurements were provided by the National Institute of Public Health and Environmental Protection. In The Netherlands, air pollution is at a low to moderate ("summer type") or a low ("winter type") level. The levels in Amsterdam and Rotterdam did not differ much for the "summer type". For 1977-89 the mean (range) values of ozone (O_3), the "summer type" pollutant (O_3-8 h), were 86 (0-252) micrograms/m^3 in Amsterdam and 82 (0-286) micrograms/m^3 in Rotterdam. The mean (range) of the values "winter type", pollutant, sulphur dioxide (SO_2-24 h), were 38 (0-381) micrograms/m^3 in Amsterdam and 50 (1-379) micrograms/m3 in Rotterdam. For black smoke (BS-24 h), values were 14 (1-84) micrograms/m^3 and 28 (1-144) micrograms/m^3 respectively (1986-89). MAIN RESULTS: Ozone had a non-significant positive effect on the number of respiratory emergency admissions in summer in people aged > or = 65 years (relative risk for a 100 micrograms/m^3 increase in O_3-8 h of 1.127 (0.983, 1.292) in Amsterdam and a significant positive effect of 1.344 (1.097, 1.647) in 1977-81 in Rotterdam). Sulphur dioxide did not show any clear effects; in Amsterdam a significant negative effect was even found. The same was true for nitrogen dioxide in Amsterdam; in Rotterdam, however, nitrogen dioxide showed non-significant positive effects (RR 0.965, 1.342). Black smoke did not show any clear effects in Amsterdam; in Rotterdam it was positively but not significantly related to the number of admissions. CONCLUSIONS: The results show that the relation between short term air pollution and emergency hospital admissions is not always consistent at these rather low levels of daily hospital admissions and of air pollution.

Objective: To assess the short term relationship between air pollution and the daily number of emergency hospital admissions for respiratory disease.

Overall study design:

Exposure level	**Exposure frequency/ duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Amerstdam: SO$_2$24 hr Mean-28µg/m^3 5th centile-3µg/m^3 95th centile-75µg/m^3 SO$_2$ 1h max	1977-1989	No info	3 groups: 0-14y 15-64y >=65y	Daily # of respiratory admissions for all ages: **Amsterdam:** Respiratory-6.7	Varied	n/a	n/a

Mean-65µg/m^3 5th centile-9µg/m^3 95th centile-167µg/m^3 **Rotterdam:** $SO_2$24 hr Mean-40µg/m^3 5th centile-5µg/m^3 95th centile-97µg/m^3 SO_2 1h max Mean-82µg/m^3 5th centile-12µg/m^3 95th centile-240µg/m^3				COPD-1.74 Asthma-1.13 **Rotterdam:** Respiratory-4.79 COPD-1.57 Asthma-0.53			

Observations:

The relative risks for a 100 µg/m^3 increase in SO_2 were calculated on hospital admissions in Amsterdam and Rotterdam. In Amsterdam, SO_2 had a positive effect on the number of respiratory admissions in the 65+ age group (RR range from 1.010-1.046). In the other age and diagnostic groups, SO_2 had a negative effect (RR from 0.802-0.995). This negative effect reached significance where asthma admissions were concerned.

In Rotterdam, respiratory admissions in the older age group were analysed for three periods: 1977-81, 1982-84 and 1985-89. SO_2 showed the same mixed results as in Amsterdam, mainly positive effects on admissions in the older age group (RR=0.892-1.258) and mainly negative effects in the other groups (RR 0.895-1.019).

SO_2 showed high levels in winter and low levels in summer. At the beginning of 1979 and the end of 1981 there were peaks in SO_2-24h exceeding 250 µg/m^3. In 1988 and 1989, SO_2 levels became very low, in winter, even below 100 µg/m^3.

*Statistically significant

Author's Conclusions:

"The results show that the relation between short term air pollution and emergency hospital admissions is not always consistent at these rather low levels of daily hospital admissions and of air pollution."

"The levels of SO_2, NO_2, and BS were lower than the levels in most cities in the studies mentioned above. The O_3 concentration was also lower but closer to the levels examined and this is the pollutant which showed the most consistent results. BS was much higher in Rotterdam than in Amsterdam. It was in Rotterdam that the most consistent results were found. This can be another indication that at these low levels of pollutants no effect on health can be found. However, other authors found effects at the same levels of air pollution as in our study. This can be explained by the number of hospital admissions in Amsterdam and Rotterdam. The total daily number of emergency admissions in these two cities was low compared with the frequency in most of the other populations."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?

G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (more info needed) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (no extrapolations made) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings for the association between SO_2 and hospital admissions for respiratory effects were inconsistent and no clear effects were present. The relative risks were close to null and all of the 95% CI included RR=1. Possible explanations are the low admission counts and the low levels of SO_2 during the study period.

This study represents one of the very few studies ever done in the Netherlands that examines the effect of air pollution on health. The past studies were conducted in the 60s, while this study focuses on April 1977-Sept 1989.

In this study, SO_2 did not show any clear effects; in Amsterdam, a significant positive effect on health was found. Results indicate that at low levels of pollutant and of low levels of hospital admissions, relation between short term air pollution and emergency hospital admissions is not always consistent.

Confidence index:

There is some information provided on the location of the monitoring stations.

The examination of missing data is detailed.

The absolute daily counts of the hospital admissions due to diseases of the respiratory system in Amsterdam and Rotterdam were low. The study may have lacked statistical power.

The standard methodology of the APHEA was followed.

The data was extensively filtered which could have weakened the association.

This study used regressive Poisson autoregression, which enabled controlling for possible confounding factors such as seasonal and other chronological factors. In addition, information such as day of admission and place of residence were recorded. The author included confidence intervals as part of the statistical analysis and results appeared to coincide with results from other APHEA findings.

Some raw data were provided, but no individual data were given.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Bacharova, L., Fandakova, K., Bratinka, J., Budinska, M., Bachar, J., Gudaba, M.	**Internal ID:** 354
Title: The association between air pollution and the daily number of deaths: findings from the Slovak Republic contribution to the APHEA project.	
Year: 1996	
Abstract: STUDY OBJECTIVE: To analyse the short term relation between daily air pollutant values and the daily number of deaths in Bratislava, Slovak Republic, during the study period 1987-91. DESIGN: This follows the APHEA protocol. The association between the daily number of total and cause specific deaths and daily variations in ambient air levels of sulphur dioxide (SO_2) and total suspended particulates (TSP) were analysed using Poisson regression that allowed control for meterological data and variables that handle temporal and autoregressive patterns. SETTING: Bratislava is the capital town of Slovakia, the total population was 442,999 according to 1991 census data. The dominant sources of industrial air pollution are the chemical industry and oil refinery. MAIN RESULTS AND CONCLUSIONS: After adjustment for season, temperature and relative humidity, days of week and holidays, secular trends, and autoregressive patterns no significant associations were found between the daily variations in the air pollutants, SO_2-24 h and TSP-24 h, and total mortality (RR 0.978, 95% CI 0.96, 0.99; RR 1.008, 95% CI 0.96, 0.99) and cause specific mortality in Bratislava during the study period	
Objective: To analyse the short term relation between daily air pollutant values and the daily number of deaths in Bratislava, Slovak Republic, during the study period 1987-91.	

Overall study design:

Exposure level	Exposure frequency/ duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
SO_2 24hr: **Season 1(winter)** Mean-42.82±47.56μg/m^3 Range-10-490μg/m^3 **Season 2(spring)** Mean-20.14±18.88μg/m^3 Range-10-180μg/m^3 **Season 3(summer)** Mean-11.86±4.99μg/m^3 Range-10-90μg/m^3 **Season 4(autumn)** Mean-20.32±16.84μg/m^3 Range-10-160μg/m^3	1987-91	No info	No info	Total number of deaths during study peirod:19062	No info	n/a	n/a

Observations:

No effect of SO_2 was apparent over the whole study period. The associated increase in SO_2 that was used to calculate the RR was not provided.

After adjustment for season, temperature and relative humidity, days of week and holidays, secular trends, and autoregressive patterns, there were no significant associations found between the daily variations in the air pollutants, SO_2-24 h, and TSP-24 hours and total mortality (RR 0.978, 95% CI 0.96, 0.99) and cause specific mortality in Bratislava during the study period

The total number of deaths during the five year period was 19062. Relatively low levels of SO_2 were observed during the study period. The mean daily concentrations measured in Bratislava exceeded the upper recommended limit according to the air quality guidelines for Slovakia in 14% of the cases.

Author's Conclusions:

"After adjustment for season, temperature and relative humidity, days of week and holidays, secular trends, and autoregressive patterns no significant associations were found between the daily variations in the air pollutants, SO_2-24 h, and total mortality (RR 0.978, 95% CI 0.96, 0.99) and cause specific mortality in Bratislava during the study period."

"Several explanations can be proposed for the lack of relation between the daily variations of air pollutants, So2 and TSP, and total and cause specific mortality in Bratislava during the study period 1987-91. Firstly, the relatively small number of inhabitants in Bratislava, resulted in small numbers of deaths per day. There are two main objections to this explanation. In comparable or even smaller towns significant associations were found…the second explanation may lie in the availability of data. Before the installation of the automated monitoring system in 1991, limited data on air pollution were available—only the mean daily concentration of SO_2 and TSP. These data might not express sufficiently the air pollution in the town. The last explanation concerns effect modifiers."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (not stated) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval?

	(n/a) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (n/a no symptom data) Was the nature and severity of symptoms graded against an explicitly defined scale? (not stated) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (more detail needed) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (no extrapolations made) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no significant findings for SO_2 and mortality of any cause. The data were inconsistent. The only significant result for SO_2 was found when comparing SO_2 levels to digestive diseases which would not be expected.

This study examined the effects of air pollution on mortality in Bratislava. While the study did not produce significant associations between these two factors, the authors did recognize several of the possible short-comings of the study, including too few deaths per day for statistical purposes. In addition, the authors suggest that insufficient data might explain the findings if SO_2 and TSP did not express sufficiently the air pollution in the town.

After adjusting for all parameters such as season, temperature, RH, days of week and holidays, no correlation was found between daily levels of SO_2 and mortality rates in Bratislava. The report gives three reasons: the sample size, the availability of data, and effect modifiers such as average life expectancy due to industrialization.

Confidence index:

More detail is needed in all areas of the study.

Very poor introduction, brief and no references cited.

The standard methodology of the APHEA was followed.

The variability and correlation between the monitoring stations was not reported.

The statistical analysis needs to be explained in greater detail.

The sample size may have been too small. The relatively small population resulted n small numbers of deaths per day which may have resulted in a lack of statistical power.

Limited data on air pollution were available prior to 1991. These data might not express sufficiently the air pollution in the town.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Ballester, F., Corella, D., Perez-Hoyos, S., Hervas, A.	**Internal ID:** 355
Title: Air pollution and mortality in Valencia, Spain: a study using the APHEA methodology	
Year:1996	
Abstract: STUDY OBJECTIVE: To assess the short term relationship between daily air pollution indicators (black smoke and sulphur dioxide (SO_2)) and mortality in Valencia. DESIGN: This was an ecological study using time series data with application of Poisson regression. Daily variations in four selected outcome variables (total mortality, mortality in those over 70 years of age, and cardiovascular and respiratory mortality) were considered in relation to daily variations in air pollution levels for the period 1991-93. SETTING: The city of Valencia, Spain. MAIN RESULTS: The mean daily mortality was 17.5, and the average daily levels of air pollutants from the three monitoring stations included in the study were, 67.7 micrograms/m^3 for black smoke, and 39.9 micrograms/m^3 for SO_2. A significant positive association between black smoke and three of the four outcomes in the study was found. The estimated relative risk (RR) of dying corresponding to a 10 micrograms/m^3 increase in mean daily black smoke over the whole period was 1.009 (95% confidence interval (95% CI): 1.003, 1.015). For mortality in the group aged more than 70 years and for cardiovascular mortality, the RRs were 1.008 (95% CI: 1.001, 1.016) and 1.012 (95% CI: 1.003, 1.022) respectively. The association with SO_2 was less clear: it was only evident during the warm season. The estimated RRs in this case were 1.007 (95% CI: 0.999, 1.015) for total mortality, 1.009 (95% CI: 1.00, 1.21) for total mortality in those older than 70, and 1.012 (95% CI: 0.995, 1.026) for cardiovascular deaths. No significant association was found between mortality from respiratory diseases and either of the two pollutants. CONCLUSIONS: A positive relationship between air pollution and mortality was found in the short term, as has been shown in an important number of studies carried out elsewhere. Although the current levels of particulate air pollution in Valencia are not very high, they could have an effect on the number of premature deaths. Despite the fact that the association is weak, it is important at the public health level both because of the numbers of population exposed and the possibility of establishing control measures.	
Objective: To assess the short term relationship between daily air pollution indicators (black smoke and sulphur dioxide (SO_2)) and mortality in Valencia.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
SO_2 24 h Mean-39.94±15.38µg/m^3 Range-7.00-105.50µg/m^3	1991-1993	No info	No info	Total Mortality-Mean17.49±5.03/day	No info	n/a	n/a

Observations:

RR were calculated for the association between increases in SO_2 of 10μg/m^3 mortality. None of the results were significant. The RR were all less than 1. These RR were all calculated for lag 2.

An association for SO_2 and mortality was only evident during the warm season. The estimated RRs in this case were 1.007 (95% CI: 0.999, 1.015) for total mortality, 1.009 (95% CI: 1.00, 1.21) for total mortality in those older than 70, and 1.012 (95% CI: 0.995, 1.026) for cardiovascular deaths. No significant association was found between mortality from respiratory diseases and either of the two pollutants.

The mean daily mortality was 17.5, and the average daily levels of air pollutants from the three monitoring stations included in the study were, 67.7 μg/m^3 for black smoke, and 39.9 μg/m^3 for SO_2. The seasonal variations in SO_2 were not large; on average, the levels in winter were 15% higher than those in summer. A significant positive association between black smoke and three of the four outcomes in the study was found. The estimated relative risk of dying corresponding to a 10 μg/m^3 increase in mean daily black smoke over the whole period was 1.009.

A significant association was found between daily mortality and the concentrations of black smoke but not for SO_2.

*Statistically significant

Author's Conclusions:

"A positive relationship between air pollution and mortality was found in the short term, as has been shown in an important number of studies carried out elsewhere. Although the current levels of particulate air pollution in Valencia are not very high, they could have an effect on the number of premature deaths. Despite the fact that the association is weak, it is important at the public health level both because of the numbers of population exposed and the possibility of establishing control measures."

"SO_2 presents less clear results in our study. It shows a seasonal pattern with a mortality association that is significant only during warm months. This fact contrasts with the results obtained in other European studies, where it emerges as the pollutant with the clearest association to mortality.

The greater effect of SO_2 on mortality during the warmer months could be a result of its greater detectability at a time when there are fewer competing risk factors. Furthermore, as there are fewer deaths during the warmer months, any variations have a greater impact on the results. However, it has been suggested that the greatest effects of pollution during hot months could be a result of higher individual exposure based on modified activity patterns."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+/-) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?

C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(n/a)** Do the conclusions make biological sense? **no extrapolations** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal?

	(no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:
The results of the SO_2 analysis were inconsistent. The results showed a seasonal pattern with a mortality association that is significant only during warm months. The results of the warm season are reported as significant; however, all of the 95% confidence intervals included RR=1. The number of deaths per day was small which may have affected the power of the study. In addition the study only spanned 3 years. The SO_2levels were low.
Air pollution is not regarded as a serious problem in Valencia and, when this study was published, no data existed on air pollution and mortality in the region. The findings were similar to those from studies carried out elsewhere. While public health associations in the Valencia findings are weak, they are still important at a public health level.

Confidence index:
Standard methodology of the APHEA project used.
Some detail provided on the calibration of the monitoring systems.
Excellent detail on the core model and confounder analysis.
Good detail on statistical analysis.
The results contained too many significant figures; in addition, results in which the confidence interval included 1 were considered significant.
The number of deaths per day was small which may have affected the power of the study. In addition the study only spanned 3 years. The SO_2 levels were also low.
The approximate locations of the monitors is not given.
This study was missing 35% of its values due to instrument malfunction at many of the monitoring stations.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Mackenbach, J.P. **Internal ID:** 356

Title: Air pollution, lagged effect of temperature, and mortality: The Netherlands 1979-87.

Year: 1993

Abstract: OBJECTIVE--To explore whether the apparent low threshold for the mortality effects of air pollution could be the result of confounding. DESIGN--The associations between mortality and sulphur dioxide (SO_2) were analysed taking into account potential confounding factors. SETTING--The Netherlands, 1979-87. MEASUREMENTS AND MAIN RESULTS--The number of deaths listed by the day on which the death occurred and by the cause of death were obtained from the Netherlands Central Bureau of Statistics. Mortality from all causes and mortality from four large groups of causes (neoplasms, cardiovascular diseases, respiratory diseases, and external causes) were related to the daily levels of SO_2 air pollution and potential confounders (available from various sources) using log-linear regression analysis. Variables considered as potential confounders were: average temperature; difference between maximum and minimum temperatures; amount of precipitation; air humidity; wind speed; influenza incidence; and calendar year, month, and weekday. Both lagged and unlagged effects of the meteorological and influenza variables were considered. Average temperature was represented by two variables--'cold', temperatures below 16.5 degrees C, and 'warm', those above 16.5 degrees C--to allow for the V shaped relation between temperature and mortality. The positive regression coefficient for the univariate effect of SO_2 density on mortality from all causes dwindles to close to zero when all potential confounding variables are taken into account. The most important of these represents the lagged (one to five days) effect of low temperatures. Low temperatures have strong lagged effects on mortality, and often precede relatively high SO_2 densities in the Netherlands. Results were similar for separate causes of death. While univariate associations suggest an effect of air pollution on mortality in all four cause of death groups, multivariate analyses show these effects, including that on mortality from respiratory diseases, are a result of confounding. CONCLUSIONS--The SO_2 density (or that of compounds closely associated with SO_2) does not seem to have any short term effect on mortality in the Netherlands. SO_2 levels higher than those currently reached in the Netherlands (above 200 micrograms/m^3) may have a measurable effect on mortality and this should be investigated. Furthermore, analyses of the public health impact of outdoor air pollution should properly control for the lagged effects of temperature.

Objective: To explore whether the apparent low threshold for the mortality effects of air pollution could be the result of confounding

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
50th centile of average SO_2density ($\mu g/m^3$); 17.86 Range:13.09-23.95	1979-87	Male and Female	No info	No clear info:	No info	n/a	n/a

Observations:

The positive regression coefficient for the univariate effect of SO_2 density on mortality from all causes dwindles to close to zero when all potential confounding variables are taken into account. The most important of these represents the lagged (one to five days) effect of low temperatures. Low temperatures have strong lagged effects on mortality, and often precede relatively high SO_2 densities in the Netherlands. Results were similar for separate causes of death. While univariate associations suggest an effect of air pollution on mortality in all four causes-of-death groups, multivariate analyses show that these effects, including that on mortality from respiratory diseases, are a result of confounding.

In a model containing temperature variables, other meteorological variables, influenza incidence, and the variables representing calendar year, month and weekday, in addition to SO_2 density, the regression coefficient for the latter variable is small and surprisingly negative, indicating lower mortality at higher air pollution levels.

*Statistically significant

Author's Conclusions:

"The SO_2 density (or that of compounds closely associated with SO_2) does not seem to have any short term effect on mortality in the Netherlands. SO_2 levels higher than those currently reached in the Netherlands (above 200 micrograms/m^3) may have a measurable effect on mortality and this should be investigated. Furthermore, analyses of the public health impact of outdoor air pollution should properly control for the lagged effects of temperature."

"We conclude that there is no evidence for a short term effect of SO_2 density (and, by implication, density of pollutants that are closely associated with SO_2) on mortality in the Netherlands. Furthermore, analyses of the public health impact of outdoor air pollution, including that by other compounds, should properly control for the important lagged effects of temperature."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(more info needed)** Is the number of subjects sufficient to produce statistically significant results? **(-)** Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? **(+)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	**(+)** Was the exposure concentration measured? **(+)** Is the exposure duration known?
C. Equipment:	**(-)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(-)** Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** ere appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(+)** Was the timing and length of follow-up appropriate?

	(+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom analysis)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? **(more info needed)** Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? **(no extrapolations made)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study is difficult to review. The results of the original association between SO_2 density and mortality were actually from a previous study by the same group. Therefore, most of the necessary details are not included in this paper. The main focus of this paper was to explore whether the apparent low threshold for the mortality effects (from the previous study) could be a result of confounding. The findings indicate that SO_2 density does not seem to have any short term effect on mortality in the Netherlands. While this study attempted to control for confounding variables, lagged effects of temperature were not controlled for. Density of SO_2 does not seem to have any short term effect on mortality in the Netherlands. However, levels measured in the Netherlands are relatively low. Further studies should be done on the effect of higher densities of SO_2 on mortality.

Confidence index:

The variation and correlation between monitoring stations is not reported.

In-depth analysis of possible confounders, except for the effect of temperature. Potential confounders were selected on the basis on their association with both mortality and SO_2 density.

According to the authors, SO_2 density is an indicator for outdoor air pollution or for the host of compounds present in polluted air. A statistical association between SO_2 density and mortality is therefore likely to be the result, at least partly of one or more of these other pollutants.

This study is consistent with all the other APHEA studies including format.

Review – Confidence index ranking:

Low to moderate

DRAFT

CASE-CONTROL REVIEW FORM

Author: Glasser, M., Greenburg, L.,	**Internal ID:** 357
Title: Air Pollution, Mortality, and Weather – New York City, 1960-1964	
Year:1971	
Abstract: Daily deaths for the years 1960 through 1964 in new York city (excluding April to September) were studied in relation to SO_2, smoke shade, temperature deviations from normal, wind speed, sky cover, and rainfall. To eliminate sesonal effects, deaths wee expressed as deviatiosnfroma five-year "normal." SO_2 was related to mortality toad greater degree when smoke shade was held constant than was smoke shade when SO_2 was held constant and was, therefore, used as an index of air pollution. Evidence suggests that this increase in mortality was associated with increased SO_2 independent of weather factors studied. The difference in mean number of daily deaths on days with mean SO_2 levels of 0.20 ppm or less compared with days having mean SO_2 levels of 0.40 ppm or more is estimated to have been 10 – 20 deaths per day.	
Objective: The objective of the present study was to investigate the relationship of all air pollution levels with mortality in New York city for a 5 year period.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Exact range of means is not clear (more detail included in observations)	5 years 1960-1964 (excluding April to September)	No specific info	No info	No info	No info	n/a	n/a

Observations:

For total study period:

The mean number of daily deaths consistently rose as the level of SO_2increased. At daily mean SO_2 values of less than 0.10ppm, the mean number of daily deaths was -3.5. Mean number of daily deaths rose to 1.8 when daily mean SO_2 levels were between 0.20-0.30ppm. There was a sharp increase to 9.4 deaths per day when SO_2 levels were between 0.30-0.40ppm. The mean number of daily deaths continued to rise slowly to 12.6 deaths per day at SO_2 levels of 0.60ppm or more. Grouping all days having less than 0.20 ppm of SO2 compared with all days having mean SO2 levels of 0.04 ppm or more, we find a difference of 15 deaths per day.

When analyzed separately by year, for three of the five years the positive relationship of daily mean SO_2 with daily mortality is discernible and statistically significant.

Evidence suggests that this increase in mortality was associated with increased SO_2 independent of weather factors studied. The difference in mean number of daily deaths on days with mean SO_2 levels of 0.20 ppm or less compared with days having mean SO_2 levels of 0.40 ppm or more is estimated to have been 10 to 20 deaths per day.

Author's Conclusions:

"We do not suggest that the relationship between air pollution and deaths here presented should by itself be used for setting allowable atmospheric standards. However, considered in contest with other information in this field we believe that this study adds useful information."

"Sulfur dioxide is related positively to daily mortality even after "adjusting," in effect, for the weather variables. The regression analysis suggests, furthermore, that the relationship of SO_2 to daily mortality is far stronger than any weather variable studied."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(more info needed)** Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptoms)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical?

	(-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-) Are the statistical methods used appropriate? (some) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (more info needed) Was the design of the study appropriate to investigate the primary objective? (more details needed) Were assumptions reasonable? (more details needed) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (no extrapolations) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study found that basically as SO_2 increased, so too did daily mortality. However, a more detailed analysis is needed to validate these results.

When this study was published, relatively little work on the relationship between day-to-day variations in air pollution with mortality during nonepisodic periods was available and few studies had considered the role of weather factors. Unfortunately, a large quantity of data was missing because of instrument breakdowns, reducing the validity of the findings.

This study's findings state that an increase in mortality was associated with increased SO_2 independent of weather factors stated. However, environmental observations reported were obtained from one sampling station which is not representative for the entire city.

Confidence index:

More detail is required on the statistical methods used.
The methods section is lacking significant detail.
The handling of weather confounders is questionable.
Only one sampling station was used for SO_2.

The relationship of SO_2 and daily deaths was calculated as the specific measure of deviation from normal used in the analysis. However, it is not clear how the 'normal' number of deaths were determined.
Weather confounders were the only confounders considered.
There is no consistency reported with other studies.
The literature review is inadequate.
This study attempted to limit possible confounders by recording climactic information daily including wind speed, sky cover, and daily rainfall. The authors felt that April through September should be omitted from the study because of the generally low levels of air pollution, thinking that data from this season would complicate the analysis. However, leaving such a large portion of the year out of analysis could introduce additional bias to the results. To minimize additional seasonal factors, the authors used transformation of data and calculated the deviations from "normal".
Raw and individual data has not been provided.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Krzyzanowski, M., Wojtyniak, B. | **Internal ID:** 359

Title: Air Pollution and daily mortality in Cracow

Year:1991/1992

Abstract: Several studies indicated that short-term increases in concentration of pollution in the atmosphere of a town may result in increased mortality in the days following the episode. To evaluate this relationship we analyzed data on daily mortality in Cracow, Poland, in the period 1977-1989 and data on daily concentrations of suspended particulates (PM_{20}) and sulfur dioxide (SO_2) in the town area. This analysis was restricted to winter months. In 1167 winter days, an average of 18.4 deaths of Cracow residents occurred daily. PM_{20} concentration exceeded 300 micrograms/m^3 in 21%, and SO_2 exceeded 200 micrograms/m^3 in 19% of those days. The correlation coefficient of the daily number of deaths with the air pollution in the 24 hours preceding the day of death was 0.093 ($p < 0.01$) for PM_{20}, and was 0.141 ($p < 0.001$) for SO_2. Similar correlations were found for air pollution levels in the four days preceding death, and the correlation decreased for earlier days. The effects were more pronounced in people over 65 years of age than in those younger, especially in males. After adjustment for SO_2 levels, PM_{20} had no additional effect on mortality. It was estimated that with an increase of SO_2 concentration of 100 micrograms/m^3, the daily number of deaths due to respiratory system diseases increased by 19% and deaths due to circulatory system diseases by 10%.

Objective: The main hypothesis tested in this study is the existence of the relation of daily number of deaths to daily air pollution levels. We shall evaluate this relationship taking into account age and sex, looking for possibly more susceptible groups of population. Further, we will consider some specific causes of death to identity groups of fatal diseases more strongly related to air pollution tan other diseases.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
SO_2exceeded 200 µg/m^3 in 6% of days. Mean levels below 40µg/m^3 in 19% of days Range of monthly means roughly:10-300µg/m^3 Maximum daily SO_2	1167 winter days	No info	No info	Average 17.3 deaths/day	No info	n/a	n/a

reached:600µg/m^3							

Observations:

In 1167 winter days, an average of 18.4 deaths of Cracow residents occurred daily. SO_2 exceeded 200 µg/m^3 in 19% of those days. The correlation coefficient of the daily number of deaths with the air pollution in the 24 hours preceding the day of death was 0.141 for SO_2. Similar correlations were found for air pollution levels in the four days preceding death, and the correlation decreased for earlier days.

For adjusted mortality, the correlations with SO_2 levels exceeded 0.13 for days with lag 0 through 3 and decreased for earlier days.

The mean number of deaths in days with SO_2 in four preceding days below 100µg/m^3 was 17.6; in days with SO_2 100-200µg/m^3, 18.3, and in days with SO_2 over 200µg/m^3, 20.0 ($p<0.01$).

Correlations of SO_2 concentrations with daily mortality were similar in males and females, as well as in people below and over 65 years of age. However, in males, the significant correlation was found only in the older group ($r=0.13$, $p<0.001$; $r=0.05$, $p>0.10$ in those younger).

Also comparison of mean number of deaths by air pollution level categories indicates stronger relation of mortality to air pollution in older people (5.8 deaths/day for SO_2 below 100µg/m^3 and 6.4 for SO_2 over 200µg/m^3 in people below 65 years of age, and 11.9 vs. 13.4 deaths in people over 65).

The relations of daily number of deaths due to "natural causes" (all except injury and poisoning), to circulatory system diseases and to respiratory system diseases to SO_2 levels (increase of 100µg/m^3) were significant as indicated by linear regression models. It was estimated that with an increase of SO_2 concentration of 100 µg/m^3, the daily number of deaths due to respiratory system diseases increased by 19% and deaths due to circulatory system diseases by 10%.

Author's Conclusions:

"The following brief conclusions may be presented:

-there is a significant correlation of daily number of deaths with air pollution levels during the four preceding days

-the mortality is related more clearly to SO2 than to PM20 concentration.

-the effect is more pronounced in people over 65 years of age than in those younger

-proportionally, the mortality due to respiratory system diseases is related to air pollution to a greater extent than deaths caused by other diseases."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?

C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-) Are the statistical methods used appropriate? (-) Are confidence intervals reported? **(some)** Did the investigator appropriately interpret the statistics? **((some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? **(more details needed)** Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(n/a)** Do the conclusions make biological sense? **(no extrapolations)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal?

	(no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

It was estimated that with an increase of SO_2 concentration of 100 μg/m^3, the daily number of deaths due to respiratory system diseases increased by 19% and deaths due to circulatory system diseases by 10%. Due to problems with the study design, further analyses are needed.

Cracow is an industrial center with major emissions of dusts and gases. Household sources, such as stoves, also contribute to air pollution. Consequently, it was prudent to conduct an air pollution study in Cracow, even though several studies had already been conducted there. Findings have health consequences as it was discovered that, in a town where SO_2 regularly exceeded 200 ug/m^3, an increase of SO_2 concentration of 100μg/m^3 could significantly increase the number of deaths due to respiratory system diseases.

Confidence index:

In the abstract, it is reported that SO_2exceeded 200μg/m^3 on 19% of the days, but in the results section it is reported as 6%.

The introduction is too brief. The literature review only consists of 3 references.

Three monitors were used for SO_2. The correlation and variability between the stations is not reported.

There is no Discussion of Results section.

Influenza epidemics are not considered in the analysis.

The figures showing pollutant levels are unclear.

Most previous studies recommend Poisson regression to analyze mortality data. This study used linear regression.

There is limited detail on the statistical analysis.

This study effectively took into consideration age and gender, looking for possibly more susceptible groups of population. However, because of instrument malfunction, the authors had to average readings from three locations in order to represent conditions in Cracow and to avoid too many days with missing data. This may have compromised the integrity of the findings.

SO_2 data was not explicitly given; only mean level was given and confidence intervals were not stated.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Touloumi, G., Pocock, S.J., Katsouyanni, K., Trichopoulos, D.	**Internal ID:** 361

Title: Short-Term Effects of Air Pollution on Daily Mortality in Athens: A Time Series Analysis

Year: 1994

Abstract: BACKGROUND. Athens has a serious air pollution problem which became evident in the early 1970s. Studies for the years 1975-1982 have indicated a positive association of sulphur dioxide (SO_2) with total daily mortality. Since 1983 the pollution profile in Athens has gradually changed but the levels of smoke, SO_2 and carbon monoxide (CO) remain relatively high. METHODS. The association of air pollution with daily all-cause mortality in Athens for the years 1984-1988 was investigated using daily values of SO_2, smoke and CO. Autoregressive models with log-transformed daily mortality as the dependent variable, were used to adjust for temperature and relative humidity (both lagged by 1 day), year, season and day of week, as well as for serial correlations in mortality. RESULTS. Graphic analysis revealed non-linear monotonically increasing relationships between total mortality and SO_2, smoke and CO, with steeper exposure-response slopes at lower air pollution levels. Air pollution data lagged by 1 day had the strongest association with daily mortality. In three separate autoregression models for log(SO_2), log(smoke) and log(CO) the regression coefficients for each were highly statistically significant ($P < 0.001$). Further multiple regression modelling showed that SO_2 and smoke are both independent predictors of daily mortality, though to a lesser extent than temperature and relative humidity. The inclusion of CO in the model did not further improve the prediction of daily mortality. The magnitude of association is small, for instance, a 10% reduction in smoke is estimated to decrease daily mortality by 0.75% (95% confidence interval [CI]: 0.51-0.99). However, it cannot be accounted for by climatic and seasonal effects, so that a causal influence of air pollution on daily mortality seems plausible. CONCLUSIONS. These findings suggest that current air pollution levels in Athens (and many other industrialized cities) may be responsible for substantial numbers of premature deaths, and hence remain an important public health issue.

Objective: The present investigation assesses the short-term health effects of air pollution indices (black smoke, SO_2 and CO on mortality from 1984 to 1988.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
$SO_2$24 hr mean: 44.92±30.16μg/m^3	1984-1988	No info	No info	Daily mortality: 37.8±12.0	No info	n/a	n/a

Observations:

Graphic analysis revealed non-linear monotonically increasing relationships between total mortality and SO_2, smoke and CO, with steeper exposure-response slopes at lower air pollution levels. Air pollution data lagged by 1 day had the strongest association with daily mortality. In three separate autoregression models for log(SO_2), log(smoke), and log(CO) the regression coefficients were highly statistically significant ($P < 0.001$). Further multiple regression modelling showed that SO_2 and smoke are both independent predictors of daily mortality, though to a lesser extent than temperature and relative humidity. A 10% reduction in SO_2 is estimated to decrease mortality by 0.65%. The regression models were repeated for each year separately. The regression coefficients were always positive and statistically significant.

The inclusion of CO in the model did not further improve the prediction of daily mortality. The magnitude of association is small, for instance, a 10% reduction in smoke is estimated to decrease daily mortality by 0.75%. However, the association cannot be accounted for by climatic and seasonal effects, so a causal influence of air pollution on daily mortality seems plausible.

Author's Conclusions:

"These findings suggest that current air pollution levels in Athens (and many other industrialized cities) may be responsible for substantial numbers of premature deaths, and hence remain an important public health issue."

"The curvilinear relations found for smoke, SO_2, and CO indicate a steeper slope at lower pollution levels than at higher levels. This is consistent with previously published results. Despite the colinearity problems which might arise from the substantial correlation among the pollutants, joint autoregressions including more than one pollutant showed that SO_2 and smoke have independent associations with mortality."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ((+) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom)

	(+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	**(more detail needed)** Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? **(more detail needed)** Were assumptions reasonable? (-) Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

In Athens, using a 5 year series of daily data, significant positive associations were observed for total daily mortality and air pollution. However, the effects were relatively small. Daily data on cause of death and age over the whole study period were not available and therefore the investigation of SO_2 and cause, as well as age-specific mortality, was not possible.

While public health measures have led to a substantial reduction in air pollution levels, there has recently been wide-spread discussion about shorter term effects of lower levels of air pollution. After controlling for confounding factors, this study suggests that low-level air pollution may have a small but real effect on mortality.

Confidence index:

Pollutants were only measured at two stations. One in the centre of town where the highest levels of SO_2 are observed. The second station was a significant

distance away from the centre of town.
A map of monitoring sites was provided. Detailed geographical distributions are not provided.
SO_2 had missing values for 1.9% of days at the Patison station and 17.4% at the Pireaus station. More information is needed on how missing values were replaced.
More information is needed on statistical models.
Confounders such as influenza epidemics were not considered.
Autoregression models were fitted to assess whether pollutants were independently predictive associations of daily mortality.

Review – Confidence index ranking:

Moderate to low

DRAFT

CASE-CONTROL REVIEW FORM

Author: Agocs, M.M., White, M.C., Ursicz, G., Olson, D.R., Vamos, A.	**Internal ID:** 362
Title: A Longitudinal Study of Ambient Air Pollutants and the Lung Peak Expiratory Flow Rates among Asthmatic Children in Hungary	
Year:1997	
Abstract: BACKGROUND: We conducted this study in Budapest, Hungary, to better characterize the effects of exposure to ambient air pollutants on the lung function of asthmatic children. METHODS: The 60 study participants were 9-14 years old, had physician-diagnosed asthma, and were symptomatic during the previous year. Their ambient air pollutant exposures to total suspended particulates (TSP) and sulphur dioxide (SO_2) were estimated from measurements made at the air monitor nearest their residence. We used analysis of variance and a fixed-effects model to assess the impact of the pollutants upon their morning and evening peak expiratory flow rates (PEFR) from 13 September to 5 December 1993. RESULTS: Total suspended particulates and SO_2 concentrations exceeded World Health Organization guideline limits on several days. Pollutant concentrations and PEFR increased during the study period. After adjusting for temperature, humidity, weekend/weekday, and the time trend, we found no consistent association between air pollutant concentrations and PEFR. CONCLUSIONS: Fall to winter seasonal changes had a large influence on PEFR and may have overshadowed the effects of the air pollutants during the study period. Seasonal influences should be carefully considered when planning future studies.	
Objective: We conducted this study to determine whether increased ambient air concentrations of total suspended particulates and SO_2 during the heating season were associated with decreased lung peak expiratory flow rages (PEFR) among a group of children with asthma in Budapest, Hungary.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Daily average (average of 8 monitoring stations) : Median- 42μg/m^3 Range-29-145μg/m^3	Sept 15-Dec 5 1993	71.7%	Median age: 11y (9-14y)	60	Varying levels of asthma	n/a	n/a

Observations:

Total suspended particulates and SO_2 concentrations exceeded World Health Organization guidelines limits on several days. No significant associations were demonstrated between pollutants and morning PEFR. However, increases in concentrations of SO_2 the previous day were significantly associated with a decreased evening PEFR: for each 10 μg/m^3 increase in the previous days' SO_2 concentration, the evening PEFR decreased an average of 0.78l/min. However, increased concentrations of SO_2 the current day were associated with a significant increase in the evening PEFR.
After adjusting for temperature, humidity, weekend/weekday, and the time trend, no consistent association was found between air pollutants and PEFR.

Author's Conclusions:

"Fall to winter seasonal changes had a large influence on PEFR and may have overshadowed the effects of the air pollutants during the study period. Seasonal influences should be carefully considered when planning future studies."
"The only significant finding was an increase in the evening PEFR as that day's SO_2 concentration increased. We then assessed the combined effects of the pollutants and found increased SO_2 concentrations were significantly associated with both an increase and decrease in evening PEFR. One would expect that an increase in pollutant concentrations would be associated with a decrease in PEFR. We did not find a consistent association between air pollutant exposure in Budapest and PEFR among asthmatic children. Given the large prevalence of asthma in this city, further research is needed to better clarify the impact of air pollution in Budapest on children's health."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more detail needed) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive?

	(n/a no symptom data) Was the nature and severity of symptoms graded against an explicitly defined scale? (-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? (+) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) **more analysis needed** Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(na/)** Do the conclusions make biological sense? **(no extrapolations)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

A consistent association between SO_2 exposure in Budapest and PEFR among asthmatic children was not observed. Problems with the study prevented an accurate analysis.

This study is relevant in that high levels of SO_2 reportedly cover 11% of the country where 44% of the population lies. Despite the relevance of the study, it had several limitations including the absence of accurate information on children's use of bronchodilators or other medication. In addition, SO_2 concentrations may have been overestimated since they were measured at monitoring stations rather than at subjects' homes.

Seasonal changes have a large influence on peak expiratory flow rates. This study suggests that this may overshadow the effects of the air pollutants during the study period. It also suggests that seasonal influences should be carefully considered when planning future studies.

Confidence index:

A training effect was not considered.

Subjects were self selected.
Exposure to environmental tobacco smoke at home was fairly common.
Significant variation between the children's mean PEFR.
Methods of detection differed among the monitors.
The amount of missing data is not provided.
The control of seasonal confounders was inadequate.
The resulting PEFR values of individual children was highly variable.
There was no blinding and many of the subjects reported believing that their illness was exacerbated by air pollution.
Only 3 statistically significant results found in 40 comparisons. This could be due to chance.
There was a lack of accurate information on children's use of bronchodilators or other medications during the study period.
Possibility of overestimating the SO_2 exposure because the concentrations were measured at the air monitor nearest to the children's residences.
This study attempted to characterize study participants, including each subject's worst season for asthma. The authors mention that three families withdrew and the rationale for exclusion. To further bolster quantitative findings of the study, the authors provided subjects with questionnaires and diaries to record demographic characteristics, activity patterns and home environment.
Confidence intervals were not reported in this study nor was there any mention of measuring devices. Characteristics of the subjects were not adequately recorded either.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Buchdahl, R., Parker, A., Stebbings, T., Babiker, A.	**Internal ID:** 364
Title: Association between air pollution and acute childhood wheezy episodes: prospective observational study	
Year:1996	
Abstract: OBJECTIVE--To examine the association between the air pollutants ozone, sulphur dioxide, and nitrogen dioxide and the incidence of acute childhood wheezy episodes. DESIGN--Prospective observational study over one year. SETTING--District general hospital. SUBJECTS--1025 children attending the accident and emergency department with acute wheezy episodes; 4285 children with other conditions as the control group. MAIN OUTCOME MEASURES--Daily incidence of acute wheezy episodes. RESULTS--After seasonal adjustment, day to day variations in daily average concentrations of ozone and sulphur dioxide were found to have significant associations with the incidence of acute wheezy episodes. The strongest association was with ozone, for which a non-linear U shaped relation was seen. In terms of the incidence rate ratio (1 at a mean 24 hour ozone concentration of 40 microg/m^3 (SD=19.1)), children were more likely to attend when the concentration was two standard deviations below the mean (incidence rate ratio=3.01; 95% confidence interval 2.17 to 4.18) or two standard deviations above the mean (1.34; 1.09 to 1.66). Sulphur dioxide had a weaker log-linear relation with incidence (1.12; 1.05 to 1.19 for each standard deviation (14.1) increase in sulphur dioxide concentration). Further adjustment for temperature and wind speed did not significantly alter these associations. CONCLUSIONS--Independent of season, temperature, and wind speed, fluctuations in concentrations of atmospheric ozone and sulphur dioxide are strongly associated with patterns of attendance at accident and emergency departments for acute childhood wheezy episodes. A critical ozone concentration seems to exist in the atmosphere above or below which children are more likely to develop symptoms.	
Objective: To examine the association between the air pollutants ozone, sulphur dioxide, and nitrogen dioxide and the incidence of acute childhood wheezy episodes.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
All seasons-1hr means:22$\pm$14μg/m^3	March 1 1992-Feb 28 1993	No info	<=16y	Cases:1025 Controls:4285	No info	n/a	n/a

Observations:

After seasonal adjustment, day to day variations in daily average concentrations of ozone and SO_2 were found to have significant associations with the incidence of acute wheezy episodes. The strongest association was with ozone, for which a non-linear U shaped relation was seen. Sulphur dioxide had a weaker log-linear relation with incidence (1.12; 1.05 to 1.19 for each standard deviation (14.1) increase in sulphur dioxide concentration). Further adjustment for temperature and wind speed did not significantly alter these associations.

Author's Conclusions:

"Independent of season, temperature, and wind speed, fluctuations in concentrations of atmospheric ozone and sulphur dioxide are strongly associated with patterns of attendance at accident and emergency departments for acute childhood wheezy episodes. A critical ozone concentration seems to exist in the atmosphere above or below which children are more likely to develop symptoms."

> "The rise in sulfur dioxide and nitrogen dioxide at the highest concentrations of ozone may occur either on hot still days or during times of stratospheric intrusions, such as thunderstorms or low pressure systems. The variations in sulfur dioxide and nitrogen dioxide on their own, however, could not explain the U shaped relation between ozone and incidence of asthma.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (more info needed)Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (more info needed) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Was the assessment identical for cases and controls? (more details needed) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (-) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (-) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals but reasons given for exclusion of data) Are all withdrawals listed, with the reason for withdrawal?

F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (more detail needed) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

After seasonal adjustment, day-to-day variations in daily average concentrations of SO_2 were found to have significant associations with the incidence of acute wheezy episodes.

This study cites the growing number of childhood asthma cases worldwide and speculates about the causation of such a phenomenon. While this study subsequently determines that concentrations of ozone above and below a critical level are associated with an increased coincidence in asthma, the author recommends that future studies focus on the whether a physiological, chemical, or biometeorological explanation can explain the occurrence.

The key messages in this study are: (1) the incidence of childhood acute wheezy episodes is greatest in the autumn and winter; (2) this study shows that after seasonal adjustment the incidence was found to be high on days when ozone concentrations were very low or very high; (3) the log incidence increased in a linear manner with increasing concentrations of SO_2.

Confidence index:

More information is needed on cases and controls.
It is not clear if controls were the same age.
Only meteorological confounders were considered.
Only one monitoring station was used for SO_2 analyses.
The significant correlations found between SO_2 and wheezy kids could be due to chance.
Different y scales of scatter plots for cases and controls.

The reliability of diagnoses may be an issue and bias the results.
Possible misdiagnosis in very young children.
This study used as controls all the other children presenting to the accident and emergency department who did not resent with symptoms of acute wheezing or shortness of breath. No mention was made of whether subjects agreed to have their data recorded as part of a study.
This study was done on children 16 years of age or under. The sample size is adequate for statistically significant data and is large enough to provide representative data for that particular demographic. Raw and individual data were not given.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Anderson, H.R., Ponce de Leon, A., Bland, J.M., Bower, J.S., Strachan, D.P.

Internal ID: 365

Title: Air pollution and daily mortality in London: 1987-1992

Year:1996

Abstract: OBJECTIVE--To investigate whether outdoor air pollution levels in London influence daily mortality. DESIGN--Poisson regression analysis of daily counts of deaths, with adjustment for effects of secular trend, seasonal and other cyclical factors, day of the week, holidays, influenza epidemic, temperature, humidity, and autocorrelation, from April 1987 to March 1992. Pollution variables were particles (black smoke), sulphur dioxide, ozone, and nitrogen dioxide, lagged 0-3 days. SETTING--Greater London. OUTCOME MEASURES--Relative risk of death from all causes (excluding accidents), respiratory disease, and cardiovascular disease. RESULTS--Ozone levels (same day) were associated with a significant increase in all cause, cardiovascular, and respiratory mortality; the effects were greater in the warm seasons (April to September) and were independent of the effects of other pollutants. In the warm season an increase of the eight hour ozone concentration from the 10th to the 90th centile of the seasonal change (7-36 ppb) was associated with an increase of 3.5% (95% confidence interval 1.7 to 5.3), 3.6% (1.04 to 6.1), and 5.4% (0.4 to 10.7) in all cause, cardiovascular, and respiratory mortality respectively. Black smoke concentrations on the previous day were significantly associated with all cause mortality, and this effect was also greater in the warm season and was independent of the effects of other pollutants. For black smoke an increase from the 10th to 90th centile in the warm season (7-19 microg/m^3) was associated with an increase of 2.5% (0.9 to 4.1) in all cause mortality. Significant but smaller and less consistent effects were also observed for nitrogen dioxide and sulphur dioxide. CONCLUSION--Daily variations in air pollution within the range currently occurring in London may have an adverse effect on daily mortality.

Objective: To investigate whether outdoor air pollution levels in London influence daily mortality.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
24hr average: 32.0±11.7μg/m^3 10th percentile- 18μg/m^3 90th percentile- 46μg/m^3	1987-1992	No info	No info	Approx. 175.5±27.0 deaths (all causes) per day	No info	n/a	n/a

Observations:

The percentage increases in daily all cause, cardiovascular, and respiratory mortality associated with increase in pollutant levels from the 10^{th} to 90^{th} percentile were calculated. A significant association between SO_2 (lag 1) ($18\text{-}46 \mu g/m^3$) and all cause mortality was observed in the warm season: RR=1.01 (1.00-1.03). However, the 95% CI includes 1.

The effects of sulfur dioxide on total mortality in the warm season remained significant after inclusion of ozone in the model but not after inclusion of black smoke or nitrogen dioxide.

Author's Conclusions:

"Daily variations in air pollution within the range currently occurring in London may have an adverse effect on daily mortality."

"These results, if indicative of a causal relation, suggest that air pollution has a measurable effect on mortality. The extent to which this is mainly due to deaths of individuals who would have died in the very near future is unclear and cannot be addressed by this type of analysis. Perhaps the main public health implication of these results lies in the possibility that much of the population is currently experiencing levels of pollution that are capable of adversely affecting the lung. Recent evidence suggests that such exposure may be associated with long term as well as short term effects on mortality. It would be prudent to assume that current levels of air pollution do have adverse health effects, and this should be taken into account in determining policies for reducing emissions of pollutants, standards for ambient concentrations, and advice to vulnerable groups."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)

E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) mortality Were assessment measures sufficiently sensitive? (n/a no symptom data) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (more detail needed) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.,* additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (no extrapolations) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings for SO_2 and daily mortality were inconsistent and not statistically significant.

This study is relevant in that while past studies dealt with the pollution generated by coal burning, currently the main sources of pollution are motor vehicles.

This study determined that much of the population might be experiencing levels of pollution that are capable of adversely affecting the lung.

This study suggests that air pollution due to particles and ozone may be associated with increased daily mortality in London. The evidence is less convincing for NO_2 and SO_2. Daily variations in air pollution within the range currently occurring in London may have an adverse effect on daily mortality.

Confidence index:
Adjusted for confounders.
Used standard methodology of the APHEA protocol.
Limited monitoring sites for the area (four) which makes it likely that exposure of the population may have been imprecisely measured.
On several occasions, one or more monitoring instruments were not operating. Conclusions focus mainly on ozone as SO_2 data was less significant.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Anderson, H.R., Spix, C., Medina, S., Schouten, J.P., Castellsague, J., Rossi, G., Zmirou, D., Touloumi, G., Wojtyniak, B., Ponka, A., Bacharova, L., Schwartz, J., Datsouyanni, K.	**Internal ID:** 369

Title: Air pollution and daily admissions for chronic obstructive pulmonary disease in 6 European cities: results from the APHEA project

Year: 1997

Abstract: We investigated the short-term effects of air pollution on hospital admissions for chronic obstructive pulmonary disease (COPD) in Europe. As part of a European project (Air Pollution and Health, a European Approach (APHEA)), we analyzed data from the cities of Amsterdam, Barcelona, London, Milan, Paris and Rotterdam, using a standardized approach to data eligibility and statistical analysis. Relative risks for daily COPD admissions were obtained using Poisson regression, controlling for: seasonal and other cycles; influenza epidemics; day of the week; temperature; humidity and autocorrelation. Summary effects for each pollutant were estimated as the mean of each city's regression coefficients weighted by the inverse of the variance, allowing for additional between-cities variance, as necessary. For all ages, the relative risks (95% confidence limits (95% CL)) for a 50 microg/m^3 increase in daily mean level of pollutant (lagged 1-3 days) were (95% CL): sulphur dioxide 1.02 (0.98, 1.06); black smoke 1.04 (1.01, 1.06); total suspended particulates 1.02 (1.00, 1.05), nitrogen dioxide 1.02 (1.00, 1.05) and ozone (8 h) 1.04 (1.02, 1.07). The results confirm that air pollution is associated with daily admissions for chronic obstructive pulmonary disease in European cities with widely varying climates. The results for particles and ozone are broadly consistent with those from North America, though the coefficients for particles are substantially smaller. Overall, the evidence points to a causal relationship but the mechanisms of action, exposure response relationships and pollutant interactions remain unclear.

Objective:
We investigated the short-term effects of air pollution on hospital admissions for chronic obstructive pulmonary disease (COPD) in Europe.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
SO_2 1h Mean: Amsterdam-50μg/m^3 Barcelona-60μg/m^3 Paris-47μg/m^3 Rotterdam-82μg/m^3	Amsterdam-1977-89 Barcelona-1986-92 Paris-1987-92 Rotterdam-1977-89	No info	No info	COPD admissions per day: Amsterdam:1.1 Barcelona:11 Paris:11 Rotterdam:1.1	Chronic obstructive pulmonary disease	n/a	n/a

Observations:

Relative risks were calculated for an increase in SO_2 of 50 μg/m^3.
The effect of SO_2 varied considerably across cities but the summary estimate was significant for the 1 h measure and borderline significant for the daily mean.

For all ages, the relative risk (95% confidence limits (95% CI)) for a 50 $\mu g/m^3$increase in daily mean level of SO_2(lagged 1-3 days) was 1.02 (0.98, 1.06).
In the warm season, significant or borderline significant effects were observed for SO_2. For a 50$\mu g/m^3$ increase in the daily mean level of SO_2 , the relative risk of COPD admission was 1.05 (1.01-1.10) in the warm season. For SO_2 1h , the relative risk was 1.02(1.00-1.04) for the warm season.
The lags were inconsistent, being either same day or day 2. This heterogeneity was largely from Amsterdam and Rotterdam; there were indications that this was associated with the use of fewer monitoring stations (one compared with three or more in the other cities) and relatively low temperatures. London, Milan, and Paris analysed the effects of 24h SO_2 on the 65 and over age group.

Author's Conclusions:

"The results confirm that air pollution is associated with daily admission for chronic obstructive pulmonary disease in European cities with widely varying climates. The results for particles and ozone are broadly consistent with those from North America, though the coefficients for particles are substantially smaller. Overall, the evidence points to a causal relationship but the mechanisms of action, exposure response relationships and pollutant interactions remain unclear."
"We have established that significant associations between air pollution and daily admissions for chronic obstructive pulmonary disease can be detected, and that among the candidate pollutants, the associations with ozone are the strongest and most consistent. Further research is required to examine interactions between pollutants, exposure response relationships and health impact. The comparatively smaller effects of particles in Europe compared with North America should be investigated further by using comparable measurements of particles and taking into account differences in the overall pollution mixture. For the present, our results indicate that current levels of air pollution are likely to be harmful to people with chronic obstructive pulmonary disease and that policies to further reduce air pollution should be continued."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+/-)may have varied for each city Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate?

	(+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) **for each city** Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? ((+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(few)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(n/a)** Do the conclusions make biological sense? **(no real extrapolations made)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Significant relative risks were associated with SO_2 and COPD for the warm season. The magnitude of the effects was small.

The results for particles and ozone are broadly consistent with those from North America, though the coefficients for particles are substantially smaller. Overall, the evidence points to a causal relationship but the mechanisms of action, exposure response relationships and pollutant interactions remain unclear.

Confidence index:
Complex seasonal patterns might not be appropriately modeled by harmonic waves.
Each centre selected the lag which gave the greatest effect.
The estimate of pollutant exposure, being based on one or several city background monitors, was likely imprecise.
Indoor levels of exposure are not considered.
The size of the relative risks is small.
Standard methodology used – APHEA protocol.
Sample size was large, but subject characterization was difficult.
The cities differed considerably in terms of size and environmental characteristics.
A wide variety of parameters were examined for their effects on health.

Review – Confidence index ranking:

Moderate to low

NON-CLINICAL REVIEW FORM

Author: Hajj, A.M., Burki, N.K., Lee, L.Y.	**Internal ID:** 370
Title: Role of tachykinins in sulfur dioxide-induced bronchoconstriction in anesthetized guinea pigs	
Year:1996	

Abstract: To investigate the role of tachykinin release in mediating the bronchoconstrictive effect of sulfur dioxide (SO_2) inhalation, measurements of dynamic lung compliance (Cdyn), total pulmonary resistance (RL), and arterial blood pressure (ABP) were made in anesthetized guinea pigs. Brief exposure (six tidal breaths) to SO_2 at concentrations between 500 and 2,000 parts/million resulted in a concentration-dependent increase in RL, decrease in Cdyn, and systemic hypotension. For example, SO_2 at 2,000 parts/million induced reversible and reproducible changes in RL, Cdyn, and ABP of 1,041 +/- 234, -60 +/- 6, and -25.8 +/- 4.3% of the baseline values, respectively. Pretreatment with two selective neurokinin- (NK) receptor (NK1 and NK2) antagonists, CP-99994 and SR-48968, resulted in almost complete inhibition of the increase in RL and of the decrease in Cdyn while preserving the decrease in ABP. Antagonism of the NK2 receptor alone resulted in inhibition of the majority of the SO_2-induced bronchoconstriction, whereas that of the NK1 and muscarinic receptors did not have a significant effect. We conclude that the release of tachykinins from sensory endings plays a central role in SO_2-induced bronchoconstriction in anesthetized guinea pigs, primarily via the activation of the NK2 receptor.

Objective: The purpose of this study was to further characterize the role of tachykinins in SO_2-induced bronchoconstriction in anesthetized vagally intact guinea pigs.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
500,1000,1500,2000 ppm	Varied per study series, not clearly stated.	Guinea pigs	Dunkin Hartley	No info	male	Study series 1: n=4 Study series 2: n=15 Study series 3: n=10 Study series 4: n=7	No info	n/a	n/a

Observations:

Study Series 1: Reproducibility of Bronchoconstrictive response to SO_2

Administration of six tidal breaths of SO_2 at four different concentrations (500-2000 ppm) induced bronchoconstriction in a concentration – dependent patter. However, different guinea pigs exhibited different degrees of responsiveness to inhaled SO_2. Specifically, the peak changes in RL (total pulmonary resistance) and in Cdyn (dynamic lung compliance) in response to 2000ppm SO_2 were 581±158 and -48.5±12.7% respectively. Repeating the SO_2 challenge set 1 h later resulted in similar concentration response curves indicating the reproducibility of bronchoconstrictive response to inhaled SO_2 in the animals.

Study Series 2: To examine the role of NK_1- and NK_2 receptor activation in SO_2 induced bronchoconstriction.
Administration of CP-99994 and SR-48968 in combination did not change baseline RL and Cdyn. However, it almost completely prevented the SO_2-induced bronchoconstriction. For instance, in the control state, inhalation of SO_2 at a concentration of 2000ppm resulted in a peak increase in RL and decrease in Cdyn of 1041±234 and -60±6% respectively. After administration of CP-00004 and SR-48968, the peak change in RL and in Cdyn after the same SO_2 2000ppm inhalation challenge were reduced to 10.2±2($p<0.01$) and -0.5±1% ($p<0.01$) respectively.

Study Series 3: To study the relative contribution of NK_1 and NK_2 receptor activation in SO_2 induced bronchoconstriction.
Administration of CP-99994
Admin. Of CP-99994 alone did not cause any inhibitory effect on the SO_2induced bronchoconstriction. The addition of SR-48968 resulted in almost complete prevention of the bronchoconstriction. For instance, in the control state, an SO_2 concentration of 2000ppm led to peak changes in RL and Cdyn of 1124±393 and -56.8±9% respectively. After admin. Of CP-99994, the pea changes in RL and Cdyn were 1119±420 and -59±12% respectively, which were not different from control responses to SO_2. After the addition of SR-48968, the peak changes in RL and Cdyn were 12.8±4.5 $p<0.01$ and -0.6±3.2% $p<0.01$ respectively.

Administration of SR48968
Admin. Of SR=48968 alone resulted in >85% reduction of SO_2 induced bronchoconstriction, and the addition of CP99994 led to an almost complete inhibition. For example, in the control state, an SO_2 concentration of 2000ppm resulted in peak changes in RL and Cdyn of 1489±528 and -59±14% respectively. After administration of SR-48968, the peak change in RL and Cdyn were significantly reduced to 168±57 $p<0.01$ and -17±6% ($p<0.01$), respectively, and after the addition of CP-99994 the peak changes in $L and Cdyn were 12.7±3.6% ($p<0.01$ compared with control and $p=0.49$ compared with after administration of SR=48968 alone) and -1.6±0.4% ($p<0.01$ compared with control and $p=0.08$ compared with after administration of SR48968 alone) respectively.\

Study Series 4: To evaluate the role of cholinergic mechanisms in SO_2induced bronchoconstriction.
Administration of atropine did not cause any change in the baseline RL and Cdyn. There was no statistically significant inhibition of SO_2induced bronchoconstriction for the whole group.
SO_2 induced bronchoconstriction in guinea pigs (n=15) was associated with a transient systemic hypotension; the mean arterial blood pressure (MABP) decreased from a baseline of 50.3±2.2 to 37.32.1mmHg at 47.1 ±8.0s after the inhalation challenge with 2000ppm SO_2. The combined administration of CP-99994 and SR48968 had no significant effect on either baseline MABP or on the SO_2 induced systemic hypotension;

Author's Conclusions:

"We conclude that the release of tachykinins from sensory endings plays a central role in SO_2 induced bronchoconstriction in anesthetized guinea pigs, primarily via the activation of the NK_2receptor."
"Tachyphylaxis to the bronchoconstrictive effects of inhaled SO_2 has been reported in both humans and guinea pigs. The diminished response to SO_2 has been reported in successive challenges was confirmed in our preliminary trials. However, we also found that the tachyphylaxis could be avoided if SO_2-challenge sets were separated by at least 1h intervals, and the bronchoconstrictive responses to inhaled SO_2 were reproducible in the individual animals... we have demonstrated that the release of tachykinins from bronchopulmonary C-fiber sensory endings plays a central role in SO_2-induced bronchoconstriction in anesthetized guinea

pigs and that the activation of the NK_2 receptor is primarily responsible for mediating this effect."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	**(-) male only** Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? **(+) guinea pig** a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* **(no info)** b) Age *Guideline: Adult, i.e.,8-12 weeks.* **(+) male** c) Sex **no info** d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* **(-) >20%**e) Weight variation *Guideline: Below 20% variation* **(no info)** f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	**(-)** Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? **(+)** Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* **(+)** Were the chosen exposure levels appropriate to investigate the primary objective? **(+)** Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(no info)** Were both nominal and actual exposure concentrations recorded? (not clearly stated) Was the duration of exposure appropriate to investigate the primary objective? **(-)** Was the duration of exposure precisely defined? **(n/a)** If exposure chambers were employed, were animals exposed during the equilibration period?

	↳ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(+)** Was the mode of administration appropriate? **(+)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(not stated)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(no info)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ↳ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(n/a)** Was the number of animals grouped in a single chamber stated? ↳ *Guideline: Groupings should permit clear observation of each animal.* **(no info)** Was the manner by which animals were grouped appropriate? ↳ *Guideline: Animals may be grouped by sex, or individually.* **(no)** Was the type and source of feed and water stated? Was it appropriate? ↳ *Guideline: Conventional lab diet* **(no)** Was the amount and feeding schedule specified? ↳ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(no)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ↳ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(-)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(n/a no exposure chamber)** Was the acclimation period specified? Was it of acceptable duration? ↳ *Guideline: 5 days* **(no)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease

	treatment, etc.) provided? **(no info)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(served as own controls)** Was a control group assayed? **(+)** Is the control regime appropriate? **(-)** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	**(+)** Were appropriate endpoints examined? **(+)** Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(-)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(+)** Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each test animal?
G. Data analysis:	**(+)**Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(+)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
H. Interpretations:	**(+)** Was the original objective addressed? **(-)** Are the results novel? **(-)** Was the design of the study appropriate to investigate the primary objective? **(-)** Were assumptions reasonable? **(-)** Are the methods reproducible? **(-)** Did either the study design or the statistical analysis limit confounding variables? **(more detail needed)** Do the conclusions make biological sense? **(no extrapolations made)** Are the extrapolations reasonable?

	(+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings showed that in anesthetized intact guinea pigs, very brief exposure to inhaled SO_2 results in a concentration-dependent bronchoconstriction and that the release of tachykinins may play a role in mediating this effect by activating NK_2receptors. The responses were reversible and reproducible.

The range of SO_2 concentrations is higher than those used in human studies and wouldn't represent exposure to the general public.

Despite allowing rest intervals of 1 hour between exposures, repeat exposures to SO_2 resulted in diminished bronchoconstriction.

Antagonism of the NK_2 receptor alone resulted in inhibition of the majority of the SO_2-induced bronchoconstriction, whereas that of the NK_1 and muscarinic receptors did not have a significant affect.

Confidence index:

There is very limited information on the test animals, including housing and feed. It was not stated whether animals were acclimated prior to exposure to SO_2.

The weight variation was greater than 20%.

The amount of anesthetic varied among the guinea pigs.

Four series of experiments were performed, with different numbers of guinea pigs in each experiment. The samples were too small in most cases and all of the animals were male.

The exposure duration is not precisely defined. Some of the study series involved the animals taking a certain number of breaths.

There was a lot of variability in response between the guinea pigs in study series 1.

There is limited information on exposure conditions and equipment.

No individual or raw data were presented for reader interpretation.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Schimmel, H., Greenburg, L.	**Internal ID:** 366
Title: A study of the relation of pollution to mortality New York City, 1963-1968	
Year:1972	
Abstract: No abstract	
Objective: The present study has as its goal the answer to the question whether well-documented estimates of excess mortality, attributable to pollution, could be obtained by studying daily mortality and pollution levels for a considerable time period, Jan. 1, 1963-Dec. 31, 1968, in a large city, New York City, where pollution ranged continuously from mild to severe.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Mean:17.25±10.73ppb	2192 days Jan. 1, 1963 to Dec.31, 1968	No info	No info	Total mortality: average 243.2 visits per day	No info	n/a	n/a

Observations:

Using an r>0.05 as criterion, it is seen that SO_2 is positively correlated with all mortality variants except infant diseases. After adjustment for temperature, the coefficients are sharply reduced."

The Estimated Average Daily Excess Deaths ranged between 18.2-36.74 with an intermediate estimate of 28.63. This latter figure would represent about 10,000 deaths a year which would not have occurred at the time they did, if there had been no pollution on the day of death or on immediately preceding days. In percentage terms, this represents 12% of the over one-half million deaths which occurred during these six years. Over 40% of the respiratory disease deaths were associated with short-term pollution effects. The coronary excess deaths associated with pollution were actually twice as large as respiratory excess deaths even though they represented only 12% of all coronary deaths. Approximately 80% of the excess deaths are attributed to the effects of smoke shade, while only 20% of the excess deaths are attributable to SO_2. These results were statistically significant. With the exception of the Tuberculosis category, the percentage attributed to smoke shade is always substantially larger than that attributed to SO2 when individual mortality categories are analyzed. SO_2 has higher partial correlation with mortality than has smoke shade.

Author's Conclusions:

"Approximately 12% of the over one half million deaths which occurred during these six years were attributable to air pollution."

"Over 40% of the respiratory disease deaths were associated with short-term pollution effects. However, the coronary excess deaths associated with pollution were actually twice as large as respiratory excess deaths even though they represented only 12% of all coronary deaths.

Here, the most we can say is that with a high degree of probability a certain portion of deaths would not have occurred at the time they did, in the absence of air pollution. This statement can only be made if fortuitous relationships are excluded."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-) Are the statistical methods used appropriate? ((-) Are confidence intervals reported?

	(more detail needed) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? **(no clear extrapolations made)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Twenty percent of excess deaths were attributable to SO_2 exposure according to the statistical analysis performed in the paper. This result is questionable due to design issues listed below.

The author suggests that air pollution may act as a stress factor, exacerbating underlying respiratory symptoms. The author recommends further studies that use data from more than one measuring station and to include data on additional pollutants.

This study relates increases in daily mortality to the short-term effects of increased pollution levels. The study goes on to point out that several improvements can be made to the experiment such as having more data or examining by individual seasons.

Confidence index:

The main weakness of the study is that only 1 monitor was used for SO_2 analysis.

The literature review consists of 2 references.

All relationships in the models were assumed to be linear.

The estimated average daily excess deaths seemed quite high.

The methods used to manipulate and analyze the data are quite complex and not clearly explained.

The excess mortality is calculated as that which is above zero pollution.

In the discussion, the authors mention that the results imply causation; however, this is a time series study.

The control of seasonal variables in the models is not well explained.

There is no information on the residence of the deceased with respect to the monitor.

Confidence intervals are not reported. Daily pollution variates were based on measurements at a single sampling station making data not very representative of the whole of New York City.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Bates, D.V., Sizto, R.	**Internal ID:** 367

Title: Air Pollution and Hospital Admissions in Southern Ontario: The Acid Summer Haze Effect

Year: 1987

Abstract: Air pollution data from 17 sampling stations between Windsor and Peterborough in Southern Ontario, for January, February, July, and August in 1974 and 1976 to 1983, have been analyzed. Each station reported O_3, NO_3, SO_2, and the coefficient of haze (COH) every hour and aerosol sulfates for a 24-hr period every sixth day using glass-fiber filters. Data on mean daily temperature and relative humidity for the region were also recorded. It is shown that there are high correlations between different pollutants and between these and temperature in the summer. In the summer, sulfate levels were significantly correlated with relative humidity. In winter, the highest correlation was between COH and NO_2. Over the 9-year period, SO_2 levels in both winter and summer have fallen considerably; there have been no significant trends in O_3, NO_3, or COH data. Aerosol sulfates increased between 1976 and 1980 in both summer and winter and have since declined slightly. Hospital admission data for the 79 acute care hospitals serving the region, which contains about 5.9 million people, have been analyzed on a daily basis for the same months of the same years. Total admissions and total respiratory admissions have declined about 15% over the period, but asthma admissions appear to have risen. The asthma category of admissions is complicated by the effects of a change in ICD coding in 1979. It has been shown that significant correlations exist between O_3, SO_4, SO_2, and temperature, on the one hand, and deviations from the mean respiratory admissions for that day of the week, for that season, for that year, on the other. These correlations exist if asthma is excluded from the diagnoses. In winter, asthma admissions are correlated with temperature only. A group of nonrespiratory conditions showed no correlations with air pollutants in winter or summer. Stepwise multiple regression analysis based on each year considered individually indicates that in summer SO_4 and temperature account for about 5% of the variance in respiratory or asthma admissions. It is shown that the mean of the hourly ozone maxima has a high correlation with the maximal 8-hr average for ozone, and that using this index instead of the mean of the hourly maxima does not increase the correlation coefficient with respiratory disease. Another analysis has been performed by grouping the hospitals and sampling stations into nine separate regions. Although a difference in respiratory admissions can be shown between high- and low-ozone days on the same day of the week, in the same season, in the same year, the relationship to respiratory disease does not appear to have been strengthened by this procedure. Recent observations have suggested he presence of unusual peaks of H^+ aerosol of small particle size in this region in the summer, concomitant with elevated O_3 and SO_4 levels. The significant of this finding in relation to the hospital admission data is not yet established.

Objective: Not clearly stated.
To look at the association between air pollution and hospital admissions in Southern Ontario.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean Hourly Peaks: Winter-2.21-5.14ppb Summer-1.65-3.97 ppb	Jan/Feb and July/Aug for 1974, 1976-83	No info	All ages	Total Admissions all causes: Winter-1,282,064 Summer-1,223456	No info	n/a	n/a

Observations:

Total respiratory admissions with and without asthma are correlated to SO_2 (lagged 48 hr) in the summer. These results are statistically significant. Asthma admissions for all ages were also correlated to SO_2 (lagged 48 hr) in the summer.

Over the 9-year period, SO_2 levels in both winter and summer have fallen considerably; there have been no significant trends in O_3, NO_3, or COH data. Aerosol sulfates increased between 197 and 1980 in both summer and winter and have since declined slightly.

Hospital admission data for the 79 acute care hospitals serving the region, which contains about 5.9 million people, have been analyzed on a daily basis for the same months of the same years. Total admissions and total respiratory admissions have declined about 15% over the period, but asthma admissions appear to have risen. The asthma category of admissions is complicated by the effects of a change in ICD coding in 1979. It has been shown that significant correlations exist between O_3, SO_4, SO_2, and temperature, on the one hand, and deviations from the mean respiratory admissions for that day of the week, for that season, for that year, on the other. These correlations exist if asthma is excluded from the diagnoses. In winter, asthma admissions are correlated with air pollutants in winter or summer.

Stepwise multiple regression analysis based on each year considered individually indicates that in summer SO_4 and temperature account for about 5% of the variance in respiratory or asthma admissions.

It is shown that the mean of the hourly ozone maxima has a high correlation with the maximal 8-hour average for ozone, and that using this index instead of the mean of the hourly maxima does not increase the correlation coefficient with respiratory disease. Another analysis has been performed by grouping the hospitals and sampling stations into nine separate regions. Although a difference in respiratory admissions can be shown between high and low-ozone days, on the same day of the week, in the same season, in the same year, the relationship to respiratory disease does not appear to have been strengthened by this procedures. Recent observations have suggested the presence of unusual peaks of H^+ aerosol of small particle size in this region in the summer, concomitant with elevated O_3 and SO_4 levels. The significance of this finding in relation to the hospital admission data is not yet established.

*Statistically significant

Author's Conclusions:

"The present data, covering 1974 to 1983, confirm and extend the general conclusion we drew previously, namely, that there is a consistent summer relationship between sulfates, ozone, and temperature and respiratory admissions, with or without asthma. This conclusion is strengthened by the continuing lack of any association of these variables with nonrespiratory conditions."

"The present data raise the question of whether the association of increased respiratory admissions in the summer in this region can be ascribed to ozone or sulfates (the only two pollutants monitored on a regular basis which show a consistent association)."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (+) Were identical 'criteria for exclusion' applied to both cases and controls?

	(+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(+) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (+) Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+/-) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	**(objective not clearly stated)** Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible?

	(+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (no clear extrapolations made) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Significant correlations exist between SO_2 and deviations from the mean respiratory admissions for that day of the week, for that season, for that year. These results are the only significant associations reported for SO_2.

The author points out that the findings may have been aggravated by the apparent increase in asthma hospital admissions since 1980. Furthermore, the author points out that hospital admission data may be unreliable when used for epidemiological studies but points out that previous studies have successfully used this method in the past.

This study concludes that there is consistent summer relationship between sulfates, ozone, and temperature and respiratory admissions, with or without asthma. However, there are not strong relationships with SO_2. This study also warns about the unreliability of hospital admission and emergency room data on the basis of misclassification.

Confidence index:

Data was available from 17 monitors and a map was provided. Admissions data was available from 79 hospitals and the locations of the hospitals were also provided on a map.

For part of the analysis, the study region is divided into 9 subregions, but it doesn't appear that SO_2 effects were analyzed.

There are references to the methodology and calibration techniques.

Nonrespiratory disease admissions are used as a control.

The analysis was complicated by the apparent increase in asthma admission since 1980. It is difficult to discern if the increase in admissions reflects a real change in the disease or a change in referral practices.

Misdiagnosis is possible with hospital admissions data.

One drawback of this study was that possible confounding factors such as climatic variables were only measured at one monitoring station, despite the large number of communities involved. However, the authors do a good job of collecting data from individuals admitted to the hospital during the sampling period, and categorizing according to causation of visit and age group.

The months chosen for data collection could be questioned. Southern Ontario has hugely varying weather patterns between May and September, and not just July and August.

Review – Confidence index ranking:

Moderate to low

NON-CLINICAL REVIEW FORM

Author: Thompson, D.C., Szarek, J.L., Altiere, R.J., Diamond, L.	**Internal ID:** 372

Title: Nonadrenergic bronchodilation induced by high concentrations of sulfur dioxide

Year: 1990

Abstract: SO_2 is an environmental pollutant known to elicit bronchospasm in susceptible subjects. We observed that brief exposure of artificially bronchoconstricted cats to high concentrations of SO_2 induces a bronchodilator response. This study assessed the characteristics of this response and examined various mechanisms that might underlie it. Cats were anesthetized with diallylbarbital-urethan, and airway smooth muscle tone, measured by lung resistance and dynamic lung compliance, was elevated with a continuous infusion of 5-hydroxytryptamine. Administration of 10 breaths of SO_2 via a tracheostomy induced concentration-dependent bronchodilation in the range 100-1,000 parts/million. Only infrequently was bronchoconstriction observed before bronchodilation. SO_2-induced bronchodilator responses were unaffected by pretreatment with intravenous atropine or propranolol, establishing them as nonadrenergic noncholinergic (NANC) in origin. Neither the ganglionic blocking agent hexamethonium nor the nerve toxin tetrodotoxin influenced the SO_2-induced bronchodilation, thus excluding a role for central or local autonomic reflexes in the response. Efforts to modulate the response by pretreatment with the cyclooxygenase inhibitor indomethacin or the mediator release inhibitor cromolyn sodium also were unsuccessful. Administration of acidic aerosols failed to mimic the SO_2-induced bronchodilator response. Although the mechanism whereby SO_2 induces bronchodilation under these experimental conditions remains unclear, release of a NANC inhibitory transmitter from a neural, epithelial, or other cellular source via a mechanism insensitive to both tetrodotoxin and cromolyn is a distinct possibility. An intrinsic NANC inhibitory system may exist in feline airways functioning as a local regulator of bronchomotor tone and possibly serving to override responses to strong, potentially asphyxial bronchoconstrictive stimuli.

Objective: We have observed that high concentrations of SO_2induce bronchodilator responses in the anesthetized cat after baseline airway tone has been elevated with an intravenous infusion of 5-hydroxytryptamine. The present study characterizes the nature of this bronchodilator response and examines various mechanisms by which it might be manifested.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
Exp.1:100,500,800,1000ppm Exp.2, 3, 4, 5,6:1000ppm	Exp.1:10 breaths per exposure level Exp.2:1, 5, 10, and 20 breaths of 1000ppm Exp.3, 4, 5, 6:4x 10 breaths	cats	No info	No info	Both m and f, but distribution not provided	No info	No info	n/a	n/a

Observations:

Administration of SO_2 to bronchoconstricted cats elicited a relaxation response that varied in duration from 2 – 10 minutes. The magnitude of this response was dose dependent. In the concentration range 100-1000ppm, significant decreases in RL were noted consistently and a significant presser response occurred after exposure to 1000ppm. However, no significant changes in Cdyn were observed.

One breath of 1000ppm SO_2 had a nonsignificant effect on RL, whereas complete reversal of induced tone was attained with 10 or 20 breaths of SO_2. Administration of 10 breaths of SO_2 via a tracheostomy induced concentration-dependent bronchodilation in the range of 100-1000 ppm.
In general, SO_2 exposure was characterized by bronchodilation alone. Bronchoconstriction was rarely observed before bronchodilation. In ~20% of the animals studied, a bronchoconstrictor phase preceded the bronchodilator phase. Small but significant increase in Cdyn and blood pressure were induced by SO_2in the range of 5-20 breaths.

Combined administration of atropine and propranolol did not significantly affect SO_2 induced bronchodilation. Blockade of ganglionic transmission with hexamethonium failed to influence the SO_2 induced bronchodilator response. Administration of tetrodotoxin induced a profound depressor response but the magnitude of the decrease in RL induced by SO_2 was unaffected by this treatment. Admnistration of acidic aerosols failed to mimic the SO_2-induced bronchodilator response.

*Statistically significant

Author's Conclusions:

"In summary, the data presented demonstrate that brief exposure to high concentrations of SO_2 induces a NANC inhibitory response in intact feline airways under conditions of enhanced bronchomotor tone The response occurs via a pathway that does not traverse a ganglionic synapse and via a mechanism that is insensitive to axonal blockade with tetrodotoxin or mediator release inhibition with cromolyn sodium. Further, the response manifests itself independently of cyclooxygenase metabolite formation and does not appear to be related to pH changes in the airways. Although the exact mechanism underlying SO_2 induced bronchodilator responses remains to be established, it may be speculated that an intrinsic NANC inhibitory system exists in the cat lung that functions as a local regulator of airway smooth muscle tone. This system may provide an important mechanism for limiting airway responses to powerful, potentially asphyxial, bronchoconstrictive stimuli."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	(no info) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* (NO)Are animal characteristics adequately recorded and appropriate for the objective of the study?

	a) Specie **(cat, no justification)** *Guideline: Rat, or justification for use of another mammalian specie.* b) Age **(no info)** *Guideline: Adult, i.e.,8-12 weeks.* **(distribution not provided)** c) Sex **(no info)** d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (-) >20% e) Weight variation *Guideline: Below 20% variation* **(no info)** f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	**(-)** Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? **(+) for Exp. 1** Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* **(+)** Were the chosen exposure levels appropriate to investigate the primary objective? **(+)** Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(no info)** Were both nominal and actual exposure concentrations recorded? **(n/a) measured as number of breaths** Was the duration of exposure appropriate to investigate the primary objective? **(-)** Was the duration of exposure precisely defined? **((no chamber)** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(-)** Was the mode of administration appropriate? **(+)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(no info)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?

C. Housing/Feeding:	**(no info)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ↳ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(n/a no chamber)** Was the number of animals grouped in a single chamber stated? ↳ *Guideline: Groupings should permit clear observation of each animal.* **(n/a)** Was the manner by which animals were grouped appropriate? ↳ *Guideline: Animals may be grouped by sex, or individually.* **(no)** Was the type and source of feed and water stated? Was it appropriate? ↳ *Guideline: Conventional lab diet* **(no)** Was the amount and feeding schedule specified? ↳ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(more info needed)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ↳ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(-)** Was a full description of the monitoring devices provided? **(not stated0** What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(n/a)** Was the acclimation period specified? Was it of acceptable duration? ↳ *Guideline: 5 days* **(no)** Was a description of the pre-test conditions (*i.e.,* diet, quarantine, disease treatment, etc.) provided? **(no info)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) **(-)** Was a control group assayed? **(n/a)** Is the control regime appropriate? **(-)** Was the period of observation following exposure appropriate? ↳ *Guideline: Longer than 14 days.*

F. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(more info needed)** Were accepted assessment methods employed? (-) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	**(limited detail)** Are the statistical methods used appropriate? (-) Are confidence intervals reported? **(more detail needed)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (-) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

SO_2expsoure was shown to elicit bronchodilation responses in the cat under conditions of elevated bronchomotor tone. The concentrations were very high (100-

1000ppm). The occurrence of bronchoconstriction was highly variable among the cats. The failure to observe a consistent bronchoconstriction response to SO_2may have been related to the mode of administration of the gas (tracheostomy) or the elevated airway tone created for the study. The results have no clinical or toxicological significance that is applicable to humans due to the test animals used and a serious lack of information and details in the study.
The significance of the results from this study is unclear because no sample size was given. The study produced inconsistent bronchoconstricter results. Future studies should attempt to limit confounding factors and clearly state sample size.
This study demonstrated that SO_2 can evoke a biphasic reflex bronchomotor response comprising an initial cholinergic bronchoconstriction followed by an adrenergic and a nonadrenergic noncholinergic bronchodilation. Further studies can be done to see whether humans posess NANC mechanisms that protect against extreme bronchoconstrictive stimuli.

Confidence index:
There is limited information on the test animas, housing/feeding and exposure conditions.
There is no justification for the use of cats.
The distribution of sexes is not given.
The weight variation was greater than 20%.
It is unclear if there was an analysis of controls
The expected response of bronchoconstriction was either not observed or very rare. This may be due to the mode of admin. (tracheostomy) or the elevated airway tone created for the purposes of the study.
The responses were highly variable among the cats.
The number of cats is not given.

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM

Author: Yokoyama, E., Yoder, R.E., Frank, N.R.	**Internal ID:** 373
Title: Distribution of ^{35}S in the Blood and its Excretion in Urine of Dogs Expose to $^{35}\text{SO}_2$	
Year:1971	
Abstract: Blood ^{35}S levels in anesthetized dogs rose progressively when the isolated upper airways were exposed to $^{35}\text{SO}_2$ for 30 to 60 minutes, and decreased slightly in the hours following exposure. The plasma contained more ^{35}S than the red blood cells (RBC); more than half of the plasma-^{35}S$^{35}\text{SO}_2$ was dialyzable, the greatest radioactivity among the nondialyzable fraction of the plasma being associated with α-globulin proteins. Two thirds of the eRBC-^{35}S appeared to be intracellular. RBC-^{35}S/Plasma^{35}S was higher following in vivo exposure of animals than when plasma^{35}S was mixed with nonradioactive RBC. Most of the urinary ^{35}S was in the form of inorganic sulfate.	
Objective: The primary purpose of our study was to extend preliminary observations that had been made on the interactions of ^{35}S and blood as well as to identify the forms in which ^{35}S is excreted in the urine. Our results are based on the exposure of both the isolated upper airways of dogs and in vitro samples of blood to $^{35}\text{SO}_2$.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
In vivo:22±2ppm and 50ppm In vitro: not clearly stated	In vivo: not clearly stated In vitro:not clearly stated **IN the abstract duration is given as 30-60min**	Dogs	No info	No info	No info	In vivo: 9	No info	n/a	n/a

Observations:

The **^{35}S in Whole Blood, Plasma, and RBC** There were no significant findings. In general, the whole blood levels rose steadily through the exposure. Four of the five animals showed a fall in ^{35}S blood levels once exposure was ended, though the fall was slight and was not necessarily progressive with time. The ^{35}S rose progressively in both plasma and RBC levels during the exposure. In the post exposure period, plasma levels tended to fall whereas the RBC levels, following the initial fall, gradually rose. The ^{35}S concentrations were always higher in the plasma than in the RBC, although the difference between concentrations was variable.

The ^{35}**S Distribution Within Plasma**

The sum of the ^{35}S in the dialyzable and non dialyzable fractions did not differ statistically from the ^{35}S measured independently in aliquots of the same **whole plasma.**

Protein Fractions

The results were variable and not statistically significant.

RBC Radioactivity

The mean percentage of radioactivity lost from the RBC through washing was 35.1% for the in vivo samples, and 63.8% for the invitro samples. The difference between the two groups was significant ($p<0.01$).

In vitro Exchange of ^{35}S Between Plasma and RBC

The ratio of RBC radioactively per plasma radioactive was significantly lower following this in vitro form of transfer than following exposure of the upper airway to **$^{35}SO_2$ (in vivo).**

Urinary Excretion of ^{35}S

^{An} average of 84.4% of the urinary ^{35}S was in the form of inorganic sulfate; 92.5% was present as total sulfate.

*Statistically significant

Author's Conclusions:

"The level of ^{35}S in the blood should reflect the balance between the rate at which ^{35}S enters the circulation, principally from the mucosa of the upper airways, and the rates at which it is excreted from the body and deposited in peripheral tissues.

We found that ^{35}S was always more concentrated in the plasma than in the RB."

"In general, we found that the radioactivity of whole blood decreased relatively little during postexposure periods lasting up to three hours despite the continuous renal excretion of ^{35}S. We found that ^{35}S was always more concentrated in the plasma than in the RBC. About one third of the radioactivity in plasma appeared to be in association with proteins, especially the alpha-globulins…the increase in RBC concentration of ^{35}S relative to the plasma concentration might reflect either a change in membrane permeability or a non-ionic binding of the ^{35}S inside the cell. On the other hand, the experiments showing that the transfer of ^{35}S from the plasma to the RBC was considerably less in vitro than in vivo are consistent with the possibility that an active transport mechanism may function during life."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	(-) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned*

	numbers should be increased. Are animal characteristics adequately recorded and appropriate for the objective of the study? **(-) dogs and no justification** a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* **(no info)** b) Age *Guideline: Adult, i.e.,8-12 weeks.* **(no info)** c) Sex **(no info)** d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* **(no info)** e) Weight variation *Guideline: Below 20% variation* **(no info)** f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	**(-)** Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? **(-)** Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* **(more info needed)** Were the chosen exposure levels appropriate to investigate the primary objective? **(-)** Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(+) in some cases** Were both nominal and actual exposure concentrations recorded? **(-)** Was the duration of exposure appropriate to investigate the primary objective? **(-)** Was the duration of exposure precisely defined? **(n/a)** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(-)** Was the mode of administration appropriate? **(-)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(no info)** Were exposure concentrations, airflow, temperature, and humidity

	monitored continuously?
C. Housing/Feeding:	**(no info)**Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? └ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(n/a no chambers)** Was the number of animals grouped in a single chamber stated? └ *Guideline: Groupings should permit clear observation of each animal.* **(no info)** Was the manner by which animals were grouped appropriate? └ *Guideline: Animals may be grouped by sex, or individually.* **(no info)** Was the type and source of feed and water stated? Was it appropriate? └ *Guideline: Conventional lab diet* **(no info)** Was the amount and feeding schedule specified? └ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(very limited)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. └ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(-)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(-)** Was the acclimation period specified? Was it of acceptable duration? └ *Guideline: 5 days* **(no)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(no info)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(no info)**Was a control group assayed?

	(n/a) Is the control regime appropriate? **(-)** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	**(-)** Were appropriate endpoints examined? **(-)** Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(more info needed)** Were accepted assessment methods employed? **(-)** Were assessment measures sufficiently sensitive? **(-)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? **(-)** Was the timing and frequency of assessment logical? **(-)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(some)** Was individual data reported for each test animal?
G. Data analysis:	**(no info)** Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(more info needed)** Did the investigator appropriately interpret the statistics? **(no)** Are the observed trends statistically significant?
H. Interpretations:	**(+)** Was the original objective addressed? **(-)** Are the results novel? **(-)** Was the design of the study appropriate to investigate the primary objective? **(-)** Were assumptions reasonable? **(-)** Are the methods reproducible? **(-)** Did either the study design or the statistical analysis limit confounding variables? **(-)** Do the conclusions make biological sense? **(-)** Are the extrapolations reasonable? **(+)** Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no findings of toxicological or clinical significance in this study. See below for an explanation.

It is unclear from these experiments whether the inspired sulfur that enters the RBC affects the cells. The amount of ^{35}S present in circulation, coincident with the peak concentration, ranged from 5% to 18% of the dose. However, numbers may not be accurate due to the inconsistencies of the dose.

Confidence index:

There is extremely limited information on the test animals, housing and feeding and equipment, exposure conditions.

The literature review is inadequate.

Clarification is needed for some of the *in vivo* methods.

It does not seem that there were controls.

For the *in vitro* experiments, the blood of unexposed dogs and humans was exposed to SO_2. The description of this analyses is extremely limited. There is no explanation for the use of the human blood and no results presented. There is no information on how many dogs provided blood, was it all from the same dog?

There was variability in the responses of the dogs.

There was no reasoning or justification given for the modes of admission, the various analyses and measurements. The results were presented but not explained.

In some of the analyses, significant results were based on the results of only 1 or 2 dogs.

Sample size was insufficient.

It was not clear whether subjects were randomly assigned to groups nor was there justification behind the different exposure levels.

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM

Author: Skornik, W.A., Brain, J.D. **Internal ID:** 374

Title:Effect of Sulfur Dioxide on Pulmonary Macrophage Endocytosis at Rest and during Exercise

Year:1990

Abstract: Inhaled SO_2 may cause damage by injuring upper airways. To what extent can SO_2 also alter pulmonary macrophage function in the parenchyma and what is the impact of exercise? We studied the effect of SO_2 on pulmonary macrophage endocytosis in resting and in exercising animals by measuring the rates of macrophage endocytosis in situ for 1 h of a test particle of insoluble radioactive colloidal gold (198Au), 1, 24, or 48 h after inhalation exposure to SO_2. Resting hamsters exposed for 4 h to 50 ppm SO_2 had no significant reduction in macrophage endocytosis compared with air-breathing control hamsters. However, if hamsters were exposed to the same concentration of SO_2 while continuously running (40 min at 0.9 km/h), macrophage endocytosis was significantly reduced 1 h after exposure even though the exposure time was only one-sixth as long. Twenty-four hours later, the percentage of gold ingested by pulmonary macrophages remained significantly depressed. By 48 h, the rate had returned to control values. Exercise alone did not affect endocytosis. Hamsters exposed to 50 ppm SO_2, with or without exercise, also showed significant reductions in the number of lavaged macrophages. This decrease was greatest and most persistent in the SO_2 plus exercise group. These data indicate that even when animals are exposed to water-soluble gases, which are normally removed by the upper airways, exercise can potentiate damage to more peripheral components of the pulmonary defense system such as the macrophage.

Objective: To study the effect of sulfur dioxide on pulmonary macrophage endocytosis at rest and during exercise.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
Exp. 1:filtered air Exp.2:50ppm Exp.3:no SO_2 Exp.4:50ppm	4 hours for Exp. 1 and 2 40 min for Exp 3 and 4	Hamsters	Syrian golden	3-5 months	Male	The analyses were run at 1h, 24h and 48h postexposure. The number of hamsters varied for each group. Exp.1: 26 Exp.2: 11(1h);13(24h); 6(48h) Exp. 3: 10(1h);6(24h);4(48h) Exp.4: 10(1h);7(24h); 4(48h)	No info	n/a	n/a

Observations:

When hamsters were exposed for a period of 4h to 50ppmSO_2, no significant reduction in macrophage endocytosis of colloidal gold was observed when compared with that in unexposed, resting hamsters. SO_2 exposed animals had phagocytic rates after a single exposure that were also identical to those in unexposed control animals. A significant increase in endocytosis was observed at 48h ($p<.0005$). After 40 min of continuous running while breathing air, there were no significant alterations in phagocytes in unexposed hamsters. However, when hamsters were exposed to 50ppm SO_2 for only 40 min while continuous exercising , macrophage endocytosis of colloidal gold was significantly reduced ($p<.0005$) 1 h after exposure . Twenty four hours later, the percent of gold ingested by pulmonary macrophages 1 h after instillation remained depressed. By 48 h postexposure, the rate of macrophage endocytosis by hamsters exposed to SO_2 while running had returned to control values. The maximum inhibition of phagocytosis was observed 1 h after exposure. The alterations in macrophage function persisted for 24 h then phagocytosis returned to control values by 48 h.

Exercise alone did not significantly change the number of recoverable pulmonary macrophages. Hamsters exposed to SO_2 , with or without exercise, showed significant reductions in the number of recoverable pulmonary macrophages. This decrease was greatest and most persistent in the SO_2 and exercise group. In contrast to the hamsters exposed to SO_2 at rest, recovery to normal macrophage levels had not begun in hamsters 48 h after exposure to SO_2while exercising.

*Statistically significant

Author's Conclusions:

"SO_2 exposure in conjunction with exercise may damage an important component of the pulmonary defense system, the alveolar macrophage. The higher flow rates typical of exercise and heavy labor result in an increased exposure of the lower airways and parenchyma to SO_2 and a decrease in the motility and/or phagocytic ability of the pulmonary macrophages . This occurred even though the duration of the SO_2 exposure was shortened from 4h to 40min. Because these important cells are the first line of defense in keeping the lungs clean and sterile, if their function is compromise the lungs may be predisposed to respiratory infection and diseases."

"We believe that increased work and activity levels can potentiate airway and parenchymal damage from inhaled toxic gases. Changes in the site of uptake, gradual use of the oral as well as the nasal pathway, and other physiologic or endocrinologic changes related to the intensity of exercise may influence the amount retained and the degree of injury that results."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	**(+) but male only** Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? **(+) hamster – no justification)** Specie *Guideline: Rat, or justification for use of another mammalian specie.* **(+)** b) Age *Guideline: Adult, i.e.,8-12 weeks.* **(male)** c) Sex **(no info)** d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* **(>20%)** e) Weight variation

	↳ *Guideline: Below 20% variation* (+) f) Source ↳ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	**(-)** Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? **(-)** Was there a gradient of exposure levels? ↳ *Guideline: Three exposure levels in addition to control.* **(no justification given)** Were the chosen exposure levels appropriate to investigate the primary objective? **(not stated)** Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(+)** Were both nominal and actual exposure concentrations recorded? **(+)** Was the duration of exposure appropriate to investigate the primary objective? **(+)** Was the duration of exposure precisely defined? **(not stated)** If exposure chambers were employed, were animals exposed during the equilibration period? ↳ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+) **except no info on lighting** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ↳ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(+/-)** Was the number of animals grouped in a single chamber stated? ↳ *Guideline: Groupings should permit clear observation of each animal.* **(+/-)**Was the manner by which animals were grouped appropriate? ↳ *Guideline: Animals may be grouped by sex, or individually.*

	(+) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (+) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (+) Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(not stated)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (-) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own

	conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	**(no info)** Are the statistical methods used appropriate? (-) Are confidence intervals reported? **(more info needed)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? **(-) some detail missing** Are the methods reproducible? **(no info)** Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? **(no extrapolations)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The responses in exposed, exercising hamsters were greater than the responses in resting hamsters. The findings suggest that the biologic effects of inhaled irritants (in hamsters) depends upon the amount and distribution of the absorbed dose which will vary depending on the exercise or activity level. The results suggest that increased work and activity levels can potentiate airway and parenchymal damage from inhaled toxic gases.

Damage occurs to alveolar macrophage when SO_2 exposure is combined with heavy exercise. These cells are "the first line of defense" in keeping lungs clean and sterile. Compromising them may cause a predisposition to respiratory infection and disease. Furthermore, exposure to SO_2 without exercise also showed significant reductions in the number of lavaged microphages.

Confidence index:

The study was well designed and the method section contained excellent detail.
There were only a few weaknesses to point out:
The hamsters were all male.
Only one exposure level was used and there was no justification for this level.

It is stated that exercising hamsters were exposed individually. However, for the rest of the experiments it is unclear. The authors also state that groups of as many as 12 resting hamsters were exposed. It is not clear if this means 'at the same time'.
Excellent literature review.
This study outlined traits of the hamsters, their housing and feeding schedules. Sample size could have been more apparent; however group size approached 12 hamsters per exposure, an adequate sample size for extrapolation of results. A control group was also used. The authors could have included raw and summarized data. In addition, no mention was made of the acclimation period.
Data collection, analysis, and interpretations were well documented and supported.

Review – Confidence index ranking:

High to moderate

DRAFT

CLINICAL REVIEW FORM

Author: Sheppard, D., Wong, W.S., Uehara, C.F., Nadel,J.A., Boushey, H.A.	**Internal ID:** 375
Title: Lower Threshold and Greater Bronchomotor Responsiveness of Asthmatic Subjects to Sulfur Dioxide	
Year:1980	
Abstract: To determine whether subjects with mild asthma or seasonal rhinitis have greater bronchomotor responses to sulfur dioxide (SO_2) than normal subjects, we undertook a study in 7 asthmatic, 7 atopic, and 7 normal subjects, 23 to 37 yr of age. We measured the change in specific airways resistance (SRaw) provoked by 10 min of breathing 1, 3, and 5 ppm of SO_2 delivered by mouthpiece on separate days at least 48 h apart. To assess the significance of parasympathetic pathways in the bronchomotor responses to SO_2, we also measured the change in SRaw provoked by 5 ppm of SO_2 in 17 of the subjects after they inhaled atropine sulfate aerosol (0.1 mg/kg). We found that in the asthmatic subjects, SRaw increased only at 5 ppm. In the asthmatic group, SRaw increased more than in either of the other groups at 5 ppm ($p < 0.005$), and was sometimes associated with marked dyspnea requiring bronchodilator therapy. The increases in SRaw produced by inhalation of SO_2 were prevented by treatment with atropine in asthmatic and nonasthmatic subjects, which suggested the involvement of parasympathetic pathways. These results indicated that subjects with mild asthma develop bronchoconstriction after exposure to concentrations of SO_2 well below currently accepted standards for occupational exposure, and that SO_2-induced bronchoconstriction is mediated by parasympathetic pathways.	
Objective: We undertook this study to ascertain whether subjects with asthma or seasonal rhinitis develop bronchoconstriction on inhaling concentrations of SO_2 that are well tolerated by normal persons, and whether the mechanisms of SO_2 induced bronchoconstriction in persons with mild asthma or seasonal rhinitis also involves parasympathetic pathways.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
1, 3, 5 ppm	10 min each	15 men 6 women	Atopic: 23-37 y Asthmatic:23-35 Normal: 24-34y	21	3 groups: atopic, asthmatic and normal	n/a	n/a

Observations:

As a group, the asthmatic subjects had significant increases in SRaw during exposure to 1, 3 and 5ppm, whereas the normal and atopic subjects had a significant increase in SRaw only at 5ppm, and this increase was significantly smaller than that seen in asthmatic subjects at 5ppm. The response in atopic subjects did not differ from the response in normal subjects at any concentration.

During exposure to 1 ppm, 4 of 7 asthmatic subjects, in of 7 normal subjects, and 1 of 7 atopic subjects developed significant increases in SRaw. During exposure of 3ppm, 7 of 7 asthmatic subjects, 1 of 7 normal subjects, and 2 of 7 atopic subjects developed significant increases in SRaw. During exposure to 5ppm, 6 of 6 asthmatic subjects, 5 of 7 normal subjects, and 5 of 7 atopic subjects had significant increases in SRaw.

Baseline values of SRaw in the 3 groups were not significantly different.

Atropine blocked the response to SO_2 in asthmatic as well as nonasthmatic subjects. 15 of 17 subjects had a significant increase after treatment with placebo. The difference between these 2 responses was significant ($p<0.0005$). The mean baseline SRaw after inhalation of atropine was significantly lower than the mean baseline value after inhalation of placebo ($p<0.025$), but in 3 subjects, baseline SRaw was almost the same after inhalation of atropine as it was after

inhalation of placebo. In all 3 subjects, SRaw increased significantly during SO_2 exposure after treatment with placebo, but not after treatment with atropine.
*Statistically significant

Author's Conclusions:

"Our finding that atropine blocked the exaggerated bronchoconstriction induced by SO_2 in all of the subjects confirmed previous studies on the effect of atropine in abolishing SO_2-induced bronchoconstriction in cats and in healthy humans, and implicated parasympathetic pathways and muscarinic receptors in the response…this study demonstrated that subjects with mild asthma develop bronchoconstriction at a lower threshold concentration of SO_2 than do nonasthmatic subjects, and that their bronchoconstriction is of greater magnitiude. In addition, the exaggerated bronchoconstriction produced by SO_2 in persons with asthma and the lesser bronchoconstriction produced by SO_2 in normal persons both appear to be mediated by parasympathetic pathways."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (+) except work history Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (more info needed) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? (+) Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (+) Were the chosen exposure levels appropriate to investigate the primary objective? (not stated) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (+) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided?

	(no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(not stated)** Were the subjects randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(+)** Was a control group assayed? **(+)** Is the control regime appropriate? **(single)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(+)** Was the length of follow-up appropriate? **(+)** Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(+)** Were appropriate endpoints examined? **(+)** Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(+)** Were accepted assessment methods employed? **(+)** Were assessment measures sufficiently sensitive? **(-)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(+)** Was the timing and frequency of assessment logical? **-)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	**(+)** Are the statistical methods used appropriate? **(+)** Are confidence intervals reported? **(+)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	**(+)** Was the original objective addressed? **(-)** Are the results novel? **(+)** Was the design of the study appropriate to investigate the primary objective? **(+)** Were assumptions reasonable? **(+) ok but a few details missing** Are the methods reproducible? **(+)** Did either the study design or the statistical analysis limit confounding variables? **(+)** Do the conclusions make biological sense? **(-)** Are the extrapolations reasonable?

	. (+) Is the paper in a peer-reviewed journal? . (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings showed that sulfur dioxide produces mild bronchoconstriction in most normal persons during brief exposure to 5ppm or greater SO_2. Bronchoconstriction developed in subjects with mild asthma at lower concentrations than it did in normal subjects or in atopic subjects. At concentrations of sulfur dioxide at which all 3 groups developed bronchoconstriction (5ppm), the bronchoconstriction response in the group of asthmatic subjects was of greater magnitude than that of the other 2 groups of subjects. These findings suggest that SO_2 induced bronchoconstriction might be clinically significant at low concentrations and that asthmatic subjects have a lower threshold and greater magnitude of response to SO_2than the normal and atopic subjects in this study.

Confidence index:

The study was well designed.
More detail is needed on the source and selection of the subjects.
It is not clear if exposure assignment was random.
There was some variability between subjects.
The use of a mouthpiece prevents inhalation by the nose. The results may have been different for nasal breathing.
The group of asthmatic subjects was small and the disease was mild. Results may have differed with subjects with more severe asthma.

Review – Confidence index ranking:

Moderate to high

CLINICAL REVIEW FORM

Author: Sheppard, D., Saisho, A., Nadel, J.A., Boushey, H.A.	**Internal ID:** 376

Title: Exercise Increase Sulfur Dioxide induced Bronchoconstriction in Asthmatic Subjects

Year: 1981

Abstract: We undertook a study to determine whether moderate exercise modifies the bronchoconstriction produced by sulfur dioxide (SO_2) in subjects with mild asthma. In 7 subjects, we compared the changes in specific airway resistance (SRaw) produced by 10 min of exercise alone (400 kpm/min on a cycle ergometer), inhalation of SO_2 alone, and the combination of exercise and SO_2. During all studies, a subject breathed SO_2 and/or air from a mouthpiece. In 6 additional subjects, we compared the increase in SRaw produced by inhalation of SO_2 during exercise with that produced by eucapnic hyperventilation with SO_2. Neither inhalation of 0.05 ppm of SO_2 at rest nor exercise or hyperventilation alone had any effect on SRaw. Inhalation of SO_2 during exercise, however, significantly increased SRaw (from 8.46 +/- 3.58 L x cm H_2O/L/s (mean +/- SD) to 18.16 +/- 10.05 at 0.05 ppm and from 8.07 +/- 2.69 to 10.48 +/- 4.49 at 0.25 ppm (p less than 0.05)). In the 2 most responsive subjects, inhalation of 0.10 ppm of SO_2 during exercise also significantly increased SRaw. The SRaw increased by the same amount whether SO_2 was inhaled during exercise or during eucapnic hyperventilation at the same minute ventilation but the time course of the increase in SRaw was different. The SRaw was at or near maximal values at the first measurement (30 s) after hyperventilation but not until 2 to 4 min after exercise. When 4 subjects took larger breaths after inhaling SO_2 during eucapnic hyperventilation to more closely match the volume of the breaths taken after exercise, the time courses of SO_2-induced bronchoconstriction after hyperventilation and after exercise were nearly identical. These results suggested that exercise increases the bronchoconstriction produced by a given concentration of SO_2 in subjects with asthma by increasing the minute volume of ventilation and that the delay in bronchoconstriction after exercise is due to the large tidal volumes that persist for some minutes during recovery.

Objective: We undertook this study to assess whether moderate exercise increases SO_2 induced bronchoconstriction in subjects with mild asthma and, if so , whether these subjects develop bronchoconstriction when they breathe concentrations of SO_2 encountered n polluted urban air. To determine whether the effects of exercise on SO_2induced bronchoconstriction are entirely a function of the increase minute ventilation associated with exercise, we also compared the bronchoconstriction caused by breathing SO_2 during exercise with that cause by performing eucapnic hyperventilation with SO_2 at a similar minute ventilation.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
First Set of Studies: Stage1-0.50ppm Stage2-0.5ppm Stage3-0.25ppm Stage4-0.10 **Second Set of Studies.** Exp.1:1ppm Exp.2:1ppm Exp.3:fltered air	First set of studies: 10 min for each exposure Second Set of Studies:5 min for each experiment	10 men 3 women	20-30 yr	13	asthmatic	No observed adverse effect level	Lowest observed adverse effect level

Exp 4: filtered air							

Observations:

In 7 subjects with mild asthma, inhalation of 0.50 and 0.25ppm of SO_2 during the performance of moderate exercise significantly increased SRaw, whereas neither inhalation of 0.50ppm of SO_2 at rest nor inhalation of humidified, filtered air during exercise had any effect on SRaw. Inhalation of 0.50ppm during exercise significantly increased SRaw from 8.46±2.69 to 18.16 +/- 10.05 in all 7 subjects ($p<0.05$). During the corresponding period of exercise alone and during inhalation of 0.50ppm at rest, SRaw did not increase in any subject. After inhalation of 0.50ppm of SO_2 during exercise, the change in SRaw was significantly greater than after exercise alone or inhalation of 0.50ppm of SO_2at rest. Inhalation of 0.25ppm during exercise significantly increased SRaw from 8.07 +/- 2.69 to 10.48 +/- 4.49 in 3 of the 7 subjects, and the increase in SRaw for the group was significant ($p<0.05$). In the two most responsive subjects, inhalation of 0.10, 0.25 and 0.50ppm SO_2 significantly increased SRaw and there appeared to be a dose response relationship.

In the second set of studies, in all 6 subjects, inhalation of 1ppm SO_2 increased SRaw both during exercise and during eucapnic hyperventilation. The magnitude of the increase in SRaw was the same when the subjects inhaled SO_2 while they exercised or while they performed eucapnic hyperventilate at the same minute ventilation. The SRaw was at or near maximal values at the first measurement (30 s) after hyperventilation but not until 2 to 4 min after exercise. When 4 subjects took larger breaths after inhaling SO_2 during eucapnic hyperventilation to more closely match the volume of the breaths taken after exercise, the time courses of SO_2-induced bronchoconstriction after hyperventilation and after exercise were nearly identical

*Statistically significant

Author's Conclusions:

"These results suggested that exercise increases the bronchoconstriction produced by a given concentration of SO_2 in subjects with asthma by increasing the minute volume of ventilation and that the delay in bronchoconstriction after exercise is due to the large tidal volumes that persist for some minutes during recovery."

"Our studies indicated that mildly asthmatic subjects develop clinically significant bronchoconstriction from very short exposures to low concentrations of SO_2. These findings suggested that the peak, rather than average concentration of SO_2 may be most important to monitor. In this study, subjects breathed through a mouthpiece, however, whereas at these exercise loads, normal persons use oronasal breathing. Because a greater percentage of SO_2 is probably removed from air inspired through the nose than that inspired through the mouth, it is conceivable that somewhat higher concentrations might be required in polluted air to produce comparable amounts of bronchoconstriction during this amount of exercise."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (more info needed) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an

	unrepresentative sample population? **(+)** Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	**(-)** Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? **(-)** Was there a gradient of exposure levels? **(+)** Were the chosen exposure levels appropriate to investigate the primary objective? **(+)** Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(+)** Were both nominal and actual exposure concentrations recorded? **(+)** Was the duration of exposure appropriate to investigate the primary objective? **(+)** Was the duration of exposure precisely defined? **(+)** Was the mode of administration appropriate? **(+)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(+)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	**(+)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined **(+)** Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(+)** Were the subjects randomly assigned to exposure groups? **(not stated)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(subjects served as own controls)** Was a control group assayed? **(+)** Is the control regime appropriate? **(+) single** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(+)** Was the length of follow-up appropriate? **(+)** Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(+)** Were appropriate endpoints examined? **(+)** Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(+)** Were accepted assessment methods employed? **(+)** Were assessment measures sufficiently sensitive? **(-)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(+)** Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(some)** Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	**(+)** Are the statistical methods used appropriate?

	(+) Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study showed that moderate exercise increases the bronchomotor effect of SO_2 in subjects with asthma so that concentrations as low as 0.10ppm can cause significant bronchoconstriction. Mildly asthmatic subjects developed clinically significant bronchoconstriction from very short exposures to low concentrations of SO_2. These results are for mouth breathing only as a mouthpiece was used to supply SO_2. Oronasal breathing of normal persons at rest would not necessarily be expected to produce the same effects at these concentrations.

These findings are relevant to normal working conditions, in that most standards allow brief exposure to SO_2 concentrations more than 10 times the concentration that caused symptomatic bronchoconstriction in this study (0.50 ppm). This suggests that SO_2 may be a significant cause of work-related exacerbations of asthma. Furthermore, since the concentrations studied are sometimes equaled or exceeded in polluted urban air, findings support the suggestion that SO_2 is at least partially responsible for the observed association between air pollution and increased morbidity from asthma.

Confidence index:

The study was generally well designed.

More detail is needed on the source and selection of the subjects.

There were an unequal amount of men and women. In the first set of studies, there was only 1 women and in the second set, only 2.

Excellent description of methods, also justification of choice of exposure levels, duration, etc.

The use of a mouthpiece might produce greater effects at lower concentrations than would be seen with oronasal breathing.

Sample size was small.

Recruitment techniques were reported, and experimental risks were communicated effectively.

Review – Confidence index ranking:

Moderate to high

CASE-CONTROL REVIEW FORM

Author: Verhoeff, A.P., Hoek, G., Schwartz, J., van Wijnen, J.H.	**Internal ID:** 377
Title: Air Pollution and Daily Mortality in Amsterdam	
Year: 1996	
Abstract: Few data are available on the association between the present low levels of air pollution in Western Europe and mortality. Daily mortality counts and the concentrations of black smoke, inhalable particles (PM_{10}), sulfur dioxide (SO_2), carbon monoxide (CO), and ozone (O_3) were available for Amsterdam from 1986 to 1992. We used Poisson regression analysis to control for seasonal and other long-term temporal patterns. Black smoke and PM_{10} were positively associated with increased risk of mortality. The relative risk for a 100-micrograms per m^3 increase in black smoke on the same day was 1.19 [95% confidence interval (CI) = 1.02-1.38], and that for a 100-micrograms per m^3 increase in PM_{10} was 1.06 (95% CI = 0.99-1.14). The relative risk for individuals over 64 years of age was higher. We found no consistent association between the levels of SO_2 or CO and daily mortality, but ozone lagged 2 days was positively associated with daily mortality. The effect of particulates on acute mortality was independent of these pollutants. The results of the present study are consistent with the relation reported between particulate air pollution and daily mortality in other communities in Europe and the United States.	
Objective: We report on the association between daily mortality and daily levels of ambient air pollution in Amsterdam.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean: $13\mu g/m^3$ Max: $139\mu g/m^3$	Study period 1986-1992	**No info**	**All ages**	**Population: 713000 in 1992**	**No info**	**n/a**	**n/a**

Observations:

We found weak associations between current-day concentrations of SO_2 or their lagged concentrations up to 2 days, and the number of daily deaths (all causes). The relative risks of death and 95% confidence interval for a $100\mu g/m^3$ were as follows: current day RR=1.042 (0.943-1.151), 1-day lag RR=1.048 (0.952-1.154) and 2-day lag RR=1.016 (0.923-1.119).

The remainder of the analysis examined the effects of SO_2 combined with other pollutants.

Author's Conclusions:

There were no conclusions made regarding SO_2 levels and daily mortality.

"The lack of association between daily mortality and 24-hour average SO_2 concentrations agrees with the results of Mackenbach et al., who reported on the relation between SO_2 concentrations and mortality in the Netherlands. It also agrees with the results of most other time-series studies considering both SO_2 and particulates and supports the findings of others that the effect of particulates on mortality is independent of SO_2".

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from

guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more information needed) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more information needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitors Was the exposure concentration measured? (+) study period 1986-1992Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (n/a) Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (n/a mortality counts) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?

G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (no conclusions re SO_2) Do the conclusions make biological sense? (no extrapolations made) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There was no significant association between SO_2 levels and daily mortality.

The effect of particulates on acute mortality was independent of these pollutants.

Confidence index:

There was no information on the cause of mortality. All causes of mortality were included.

Only the mean SO_2 concentration for the whole study period was reported. The standard deviations were also not provided.

SO_2 was measured continuously at 11 different sites within the city. However, there is very little information provided regarding the measurements, homogeneity between sites etc.

The method section was very detailed. All methods of analysis were justified and references given to other studies.

Many confounders were considered in the analysis. This study did not consider some confounding factors that might have had a significant impact on the results, including pre-existing health conditions such as respiratory symptoms and lung function of its sample subjects.

Review – Confidence index ranking:

Moderate to low

NON-CLINICAL REVIEW FORM

Author: Meng, Z., Zhang, B., Ruan, A., Sang, N., Zhang, J. **Internal ID:** 380

Title: Micronuclei induced by sulfur dioxide inhalation in mouse bone marrow cells in vivo

Year: 2002

Abstract: Induction effects of sulfur dioxide (SO_2) inhalation on micronuclei (MN) formation in the polychromatic erythrocytes (PCE) of mouse bone marrow were studied in vivo. It was shown that SO_2 inhalation caused an increase of MN frequencies in the PCE cells in a dose-dependent manner. These results from male and female mice were very similar; no sex difference was observed. These results imply that SO_2 is a clastogenic and genotoxic agent.

Objective: In the present study, we evaluated SO_2 inhalation for their ability to induce MN in the polychromatic erythrocytes (PCE) of mouse bone marrow in vivo.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
14, 28, 56, 84 mg/m^3	4hrs/d for 7days	Mice	Kunming	6 wks	M and F	100 mice (10 F and 10 M per experiment)			

Observations:

The control group resulted in a background level of 0.113±0.10% and 0.125±0.09% micronuclei polychromatic erythrocytes (MNPCE) in female and male mice, respectively. For both male and female mouse bone marrow cells, the frequencies of MNPCE increased significantly with dosage of SO_2. The responses of male and female mice to SO_2were very similar. SO_2 inhalation increased the frequencies of PCE with bimicronuclei for the treatments at higher dosages (56, 84 mg/m^3) in a dose dependent manner. There was no increase in PCE with triMN at all concentrations tested.

Author's Conclusions:

"Inhalation of SO_2 might impair the deformability of erythrocytes by increasing associations between hemoglobin and membrane proteins. Results imply that SO_2 is a clastogenic and genotoxic agent."

"Although our data are still insufficient, this is the first experimental evidence that SO_2 inhalation induces MN in mouse bone-marrow cells in a dose-dependent manner in vivo. These results have confirmed our previous observations that SO_2 is a clastogenic and genotoxic agent. Several possibilities remain to explain the mechanism of SO_2-induced MN formation. It is possible the bisulfite induces chromosomal damage via DNA modification and deamination of cytosine to uracil, an enzyme-catalyzed process that is proposed for mammalian cells. A second possible mechanism for SO_2-induced chromosomal damage may involve formation of sulfur- and oxygen-centered free radicals."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from

guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* **(-) 6 weeks** b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex **(no info)** d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (+) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(+) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? **(no info)** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+)Was the mode of administration appropriate?

	(+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? (-) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? **(+)** *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(no)** Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* **(no info)** Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (+) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (+) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(+) just dimensions** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-) Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (-) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+) Were test animals randomly assigned to exposure groups? **(no)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random

	assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? **(sacrificed after 24 hours)** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) **yes, for the objective of the study** Were appropriate endpoints examined? (+) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (more info needed) Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? **(no extrapolations)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The preliminary results suggest that SO_2 may be a clastogenic and genotoxic agent in mice. More studies are needed to confirm these findings as, according to the authors, SO_2 inhalation-induced MN and its dose-effect relationship have not previously been carried out in vivo.

Confidence index:

The mice were only 6 weeks old.
There is limited information on the monitoring devices.
There is no information on acclimation or equilibration periods.
SO_2 was not continuously measured.
Results from this study are very preliminary. Since this was the first study of its kind, preliminary trials to determine exposure levels and evidence to justify them should have been stated but were not. Also, sensitivity of the monitoring devices was not employed, and confidence levels were not given. Sample size was not given and so it could not be determined whether results are statistically significant.

Review – Confidence index ranking:

Low to moderate

NON-CLINICAL REVIEW FORM

Author: Meng, Z.	**Internal ID:** 381

Title: Oxidative Damage of Sulfur Dioxide on Various Organs of Mice: Sulfur Dioxide is a Systemic Oxidative Damage Agent

Year: 2003

Abstract: Cu,Zn-superoxide dismutase (SOD), Se-dependent glutathione peroxidase (GSH-Px), catalase (CAT), and glutathione (GSH) play an important role in attenuating free radical-induced oxidative damage. The purpose of this research was to determine (1) whether sulfur dioxide (SO_2) increases levels of lipid peroxidation and alters intracellular redox status in multiple organs of mice, and (2) whether SO_2 is a systemic toxic agent. The effect of SO_2 on levels of thiobarbituric acid-reactive substances (TBARS) and GSH and activities of SOD, GSH-Px, and CAT were investigated in nine organs (brain, lung, heart, liver, stomach, intestine, spleen, kidney, and testis) of Kunming albino mice of both sexes. SO_2 at 20 ppm (56 mg/m^3) was administrated to the animals of SO_2 groups in an exposure chamber for 6 h/day for 7 days while control groups were exposed to filtered air in the same condition. Results show that SO_2 inhalation decreased significantly activities of SOD and GSH-Px in all organs tested in all $_{SO_2}$ groups, with respect to their corresponding control groups; CAT activities in all organs tested of both sexual mice were significantly unaltered, except CAT activities in livers were significantly lowered by SO_2; SO_2 exposure decreased significantly GSH contents and significantly increased TBARS levels of all organs tested, in comparison with their respective control groups. These results lead to two conclusions: (1) $_{SO_2}$ is a systemic oxidative damage agent. It results in a significant increase in the lipid peroxidation process in all organs tested of mice of both sexes, which is accompanied by changes of antioxidant status in these organs. (2) SO_2 may cause toxicological damage to multiple organs of animals, and it is suggested that the oxidative damage produced by SO_2 inhalation may influence or promote the progression or occurrence of some disease states of various organs, not only to respiratory system. Further work is required to understand the toxicological role of SO_2 on multiple or even all organs in mammals.

Objective: In the present study, nine organs (brain, lung, heart, liver, stomach, intestine, spleen kidney, and testis) of mice were assessed for oxidative stress and antioxidant status caused by SO_2 inhalation.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
20ppm	6h/day 7 days	Mice	Kunming albino	5 wks	50%F 50%M	N=40 20 control and 20 exposed	No info	n/a	n/a

Observations:

SO_2 inhalation caused statistically significant decreases of SOD activities in all organs tested in mice of both sexes. However, SO_2 yielded statistically more significant deceases of SOD activities in the brain, lung, stomach, and intestine from male animals and in lung, heart, liver, intestine, and kidney from female.

SO_2 inhalation significantly decreased activities of GSH-Px (Se-dependent) in all organs tested in mice of both sexes.

SO_2 inhalation caused a significant decrease of CAT activities in livers from male and female mice. CAT activities of all other organs tested in mice of both sexes were significantly unaltered in all experimental groups after SO_2expousre compared with their respective control groups.

SO_2inhalation significantly decreased GSH levels in all organs tested of both sexual mice. The changes of GSH/GSSG in all organs observed of both male and female mice were not statistically significant in all experimental groups after SO_2 exposure compared with their respective control groups.
TBARS levels in all organs tested in mice of both sexes were significantly increase in all SO_2 exposed groups compared with controls.

Author's Conclusions:

"In this study the significant changes of oxidative stress and antioxidant status in all organs tested in SO_2 groups are not unexpected. It is known that SO_2 is a systemic oxidative damage agent to animals, not only to respiratory systems. Therefore, it is suggested that SO_2 may cause toxicological damages to multiple organs of animals, and the oxidative damage produced by SO_2 or its derivatives may influence or promote the progression or occurrence of some disease states of various organs, not only to respiratory system. Further work is required to understand toxicological role of SO_2 on multiple or even all organs in mammals."
"The present results show that SO_2 inhalation increased levels of lipid peroxidation in all organs tested in mice of both sexes, accompanied by a significant decrease of the activities of Cu, Zn-SOD and Se-dependent GSH-Px and levels of GSH in all organs tested."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (-) **5 weeks** b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex **(no info)** d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* (+) e) Weight variation *Guideline: Below 20% variation* (+) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels?

	↳ *Guideline: Three exposure levels in addition to control.* **(+)** Were the chosen exposure levels appropriate to investigate the primary objective? **(-)** Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(not stated)** Were both nominal and actual exposure concentrations recorded? **(+)** Was the duration of exposure appropriate to investigate the primary objective? **(+)** Was the duration of exposure precisely defined? **(limited info)** If exposure chambers were employed, were animals exposed during the equilibration period? ↳ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(+)** Was the mode of administration appropriate? **(+)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(not stated)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(+)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ↳ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(yes, 10 per cage, but no info on how many in exposure chamber)** Was the number of animals grouped in a single chamber stated? ↳ *Guideline: Groupings should permit clear observation of each animal.* **(+)** Was the manner by which animals were grouped appropriate? ↳ *Guideline: Animals may be grouped by sex, or individually.* **(+)** Was the type and source of feed and water stated? Was it appropriate? ↳ *Guideline: Conventional lab diet* **(+)** Was the amount and feeding schedule specified? ↳ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water*

	may be withheld optionally.
D. Equipment:	**(just dimensions)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-) Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* (+) Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? (+) Were test animals randomly assigned to exposure groups? **(not stated)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? **(n/a)** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) **for the objective of the study** Were appropriate endpoints examined? (+) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?

H. Interpretations:	**(+)** Was the original objective addressed? **(+)** Are the results novel? **(+)** Was the design of the study appropriate to investigate the primary objective? **(+)** Were assumptions reasonable? **(-)** Are the methods reproducible? **(+)** Did either the study design or the statistical analysis limit confounding variables? **(more info needed)** Do the conclusions make biological sense? **(no extrapolations)** Are the extrapolations reasonable? **(+)** Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The study findings suggest that SO_2 inhalation increases levels of lipid peroxidation in all organs tested in mice of both sexes accompanied by an significant decrease of the activities of Cu,Zn-SOD and Se-dependent GSH-Px and levels of GSH in all organs tested. These results provide evidence that SO_2may be a systemic oxidative damage agent and may cause toxicological damage to multiple organs in mice and further studies are warranted.

Animals were subjected to regular periods of extended exposure, with relief periods between protocols. This may provide a corollary to individuals exposed to the gas in an occupational setting. This study concludes that SO_2 inhalation damages various organs as well as the respiratory system. Also, a significant decrease in Cu,Zn-SOD might predispose organs to increased free-radical damage because SOD can catalyze decomposition of superoxide radicals to produce hydrogen peroxide.

Confidence index:

Extensive literature review.

Excellent discussion of results.

More detail is needed on exposure conditions. There is no information on equilibration periods or acclimation.

Subjects were not well-characterized with respect to health status.

The authors limited confounding factors by choosing mice that were all 5 weeks old and who had a weight variation of approximately 20%.

Mice were randomly divided into groups. Water and feeding schedules were described.

Raw and individual data were not provided.

Review – Confidence index ranking:

Moderate to low

CASE-CONTROL REVIEW FORM

Author: **Romieu, I., Meneses, F., Sienra-Monge, J.J.L., Huerta, J., Velasco, S.R., White, M.C., Etzel, R.A., Hernandez-Avila, M.**

Internal ID: 385

Title: **Effects of Urban Air Pollutants on Emergency Visits for Childhood Asthma**

Year: **1995**

Abstract: The metropolitan area of Mexico City, Mexico, has serious air pollution problems. Although air contaminants may contribute to clinical asthma, there are at present no data on the relation between air pollution exposure and childhood asthma in Mexico City. The authors reviewed data on emergency visits from January to June 1990 at one major pediatric hospital in Mexico City. They used a Poisson regression model to study the relation between the number of daily emergency visits for asthma and air pollutant levels. The levels of ozone and sulfur dioxide-exposure were significantly associated with the number of emergency visits for asthma. After adjustment for potential confounding factors, the multivariate regression model predicted that an increase of 50 ppb in the 1-hour maximum ozone level would lead to a 43% increase in the number of emergency visits for asthma on the following day. Exposure to high ozone levels (> 110 ppb) for 2 consecutive days increased the number of asthma-related emergency visits by 68 percent. The results of this study suggest that ozone exposure is positively associated with the number of children's emergency visits for asthma in Mexico City.

PIP: A retrospective analysis of records of emergency visits during January-June 1990 at the Hospital Infantil de Mexico Federico Gomez in the northern section of Mexico City was conducted to examine the association between the 395 children's (16 years) emergency visits for asthma (2.5% of all emergency visits) and daily levels of different air pollutants. Most emergency visits for asthma (67%) included children aged less than 5. The mean ozone daily one-hour maximum level was 90 ppb (range: 10-250 ppb). The ozone level exceeded the Mexican ozone standard (110 ppb) on 49 days (28%). Concentrations of ozone and sulfur dioxide exposure were linked to the number of emergency visits for asthma ($p = 0.001$ for 1-hour maximum with 1-day lag and $p = 0.07$ for 1-hour maximum on same day). When the researchers controlled for confounding factors, they found that a rise in the ozone level of 50 ppb was related to a 43% increase in the number of emergency visits for asthma with a one-day lag period. 133% of these visits were made after two consecutive days of ozone levels of at least 110 ppb. Exposure to such high ozone levels increased the number of emergency visits for asthma by 68%. These findings demonstrate a positive relationship between ozone exposure and the number of children living in Mexico City who were treated for asthma. In fact, they suggest that cumulative exposure to ozone strengthens this association.

Objective: In this report, we present results from a study conducted in a large pediatric hospital in Mexico City to determine the relation between the number of children's emergency visits for asthma and the daily levels of different air pollutants.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Mean: 0.07 ppm Range: 0.01-0.49ppm	Study period: January – June 1990	No info	<16 years	Population of children approx. 450,000	No info	n/a	n/a

Observations:

N=1365 of the patients sought treatment for respiratory conditions; N=395 of this group had conditions related to asthma.

Levels of sulfur dioxide (daily 1-hour maximum) were related to the number of emergency visits for asthma (not statistically significant) and to the total number of emergency visits for respiratory disease (statistically significant) on the same day, but were not related to the total number of emergency visits. An increase of 50 ppb in the daily mean sulfur dioxide level led to increases of 5% in the number of emergency visits for asthma on the same day (these results were not statistically significant).

SO_2 level exceeded the standard guideline of 0.13ppm on several occasions. Peak levels were usually observed around 9am. The effect of SO_2 on asthma related visits was only marginally significant when the other variables were included in the model.

Author's Conclusions:

"Sulfur dioxide concentrations on the concurrent day were related to an increase in respiratory-related emergency visits, including asthma. This has also been observed in other studies. Sulfur dioxide is known to be an irritant to the respiratory tract, and asthmatic patients exposed to sulfur dioxide in controlled human studies experience higher decreases in lung functions than do healthy individuals. However, when ozone and temperature levels were included in our regression model, the effect of sulfur dioxide on asthma-related emergency visits was small."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more information needed) Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Fixed monitorsWas the exposure concentration measured? (+) Study period 6 monthsIs the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (No info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (-) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (more info needed) Were appropriate endpoints examined?

	(more info needed) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(more info needed)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (-) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) **more info needed** Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(n/a no specific conclusions reSO_2)** Do the conclusions make biological sense? **(no extrapolations)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The levels of sulfur dioxide were reported to be significantly associated with the number of emergency visits for asthma. However, the results are not statistically significant. The association between levels of sulfur dioxide and respiratory admissions appears to be significant (p=0.002); however, relative risks and confidence intervals are not given.

Confidence index:

The introduction is very brief with few references. There is no specific mention of SO_2, just ozone.

Possible misdiagnosis in very young children, <1year..
The population is difficult to quantify because patients are not assigned to a specific health care unit and because the Hospital is a referral hospital.
The study period was only 6 months long.
There was only one monitor to measure SO_2 There are no details given on the measurement of SO_2.
The results of this study would not be generalizable to other populations.
According to the authors, in Mexico, the first episode of asthma among children is usually, but not always, diagnosed as bronchiolitis and therefore these cases were included as well.
More detail is needed on the calculation of statistical significance and the methods for including confounders into the study.
The discussion briefly mentions SO_2, but otherwise the focus of the study is on the effects of ozone.
In the discussion, sulfur dioxide concentrations on the concurrent day are reported to be related to an increase in respiratory related emergency visits, including asthma, but reference is given to only one study.
In the abstract, the association between levels of SO_2 and asthma are reported to be significant; however, in the paper these results are not statistically significant. Rate ratio is 1.05 and 95% CI is 0.99-1.11. P values are not provided.
This study was done over a period of only 6 months from January to June. Longer-term studies should be done as well as follow ups to gain more insight into exposure. Also, asthma attacks may have been misclassified because of the variety of clinical symptoms that asthma produces. As with all population studies, there is uncertainty about individual exposure and the inability to control for confounders.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Morris, R.D., Naumova, E.N., Munasinghe, R.L.	**Internal ID:** 387

Title: Ambient Air Pollution and Hospitalization for Congestive Heart Failure among Elderly People in Seven Large U.S. Cities

Year: 1995

Abstract: OBJECTIVES. Preexisting data sets were used to investigate the association between hospital admissions for congestive heart failure and air pollutants. METHODS. Medicare hospital admissions data, ambient air pollution monitoring data, and meteorological data were used to create daily values of hospital admissions for congestive heart failure, maximum hourly temperature, and maximum hourly levels of carbon monoxide, nitrogen dioxide, sulfur dioxide, and ozone. Data were compiled for each of seven cities (Chicago, Detroit, Houston, Los Angeles, Milwaukee, New York, and Philadelphia) for 1986 through 1989. Single-pollutant and multipollutant models with adjustments for temperature, seasonal effects, and weekly cycles were used in conducting negative binomial regression analyses. RESULTS. Ambient carbon monoxide levels were positively associated with hospital admissions for congestive heart failure in the single-pollutant and multipollutant models for each of the seven cities. The relative risk of hospital admission for congestive heart failure associated with an increase of 10 ppm in carbon monoxide ranged from 1.10 in New York to 1.37 in Los Angeles. CONCLUSIONS. Hospital admissions for congestive heart failure exhibited a consistent association with daily variations in ambient carbon monoxide. This association was independent of season, temperature, and other major gaseous pollutants.

Objective: Preexisting data sets were used to investigate the association between hospital admissions for congestive heart failure and air pollutants.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Mean SO_2 levels: 0.010-0.032 ppm	Study period: 1986-1989	No info	$\geq$65 years	Total Admissions for Congestive Heart Failure for the 7 U.S. cities ranged from: 10819-57671	No info	**n/a**	**n/a**

Observations:

Values refer to the relative risk associated with an increase of 0.05 ppm SO_2. The relative risks for the seven cities ranged from 0.95-1.41. The confidence intervals included RR=1 for 5 of the cities.

Author's Conclusions:

There were no specific conclusions relating SO_2levels and congestive heart failure.

"In summary, this study demonstrates a clear and consistent association between ambient levels of carbon monoxide and hospital admissions for congestive heart failure among elderly people. This effect does not appear to result from the confounding effect of weather or other seasonal factors. Although nitrogen dioxide, sulfur dioxide, and ozone do not explain the apparent effect of carbon monoxide, it is possible that the observed association represents the impact of some other, unmeasured pollutant or group of pollutants covarying in time with carbon monoxide."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitors Was the exposure concentration measured? (+) study period 4 years Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (more info needed) Were appropriate endpoints examined? (more info needed) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (more info needed) Were accepted assessment methods employed?

	(more info needed) Were assessment measures sufficiently sensitive? **(more info needed)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	**(not clearly)** Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? **(some)**Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(no specific conclusions made)** Do the conclusions make biological sense? **(no extrapolations made)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(No)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Only the results for New York and Los Angeles were statistically significant. New York had the highest average values of SO_2; Los Angeles had the lowest. New York had the third highest number of cases, whereas Los Angeles had the most cases. The results regarding SO_2 levels were inconsistent.

This study was somewhat unique in that it focused on cardiovascular rather than respiratory health effects associated with air pollution.

This study presents clear and consistent association between CO and congestive heart failure in the elderly. No obvious conclusions were made about SO_2. There should be further understanding of the factors responsible for the observed increases in hospital admissions for congestive heart failure to develop sound strategies for public health intervention.

Confidence index:

The results section discusses the results of CO analysis only.

The literature review is inadequate.

There is an insufficient amount of information on the monitoring techniques and sites.

This study limited participants to those with a primary discharge diagnosis of congestive heart failure. Furthermore, the database contained information on age, sex, race, zip code of residence and data of admission of the patient.

This is a thorough study with good procedure, data analysis, and well supported interpretations; however conclusions are mainly drawn from the observed effects of carbon monoxide.

Review – Confidence index ranking:

Moderate to low

DRAFT

CASE-CONTROL REVIEW FORM

Author: Ponka, A., Virtanen, M.	**Internal ID:** 388
Title: Low-Level Air Pollution and Hospital Admissions for Cardiac and Cerebrovascular Diseases in Helsinki	
Year: 1996	
Abstract: OBJECTIVES: This study investigated whether low concentrations of ambient air pollutants are associated with hospital admissions for ischemic cardiac and cerebrovascular diseases. METHODS: Associations between daily concentrations of sulfur dioxide, nitric oxide, nitrogen dioxide, ozone, and particulates and daily hospital admissions due to ischemic cardiac and cerebrovascular diseases were studied in Helsinki, Finland, 1987 through 1989. The regression analyses controlled for weather, day of the week, season, long-term trends, and influenza epidemics. RESULTS: Admissions via emergency rooms due to ischemic cardiac diseases (n = 7005) were significantly associated with the prevailing levels of nitric oxide and ozone, and those due to cerebrovascular diseases (n = 3737) were associated with nitrogen dioxide; these levels were only moderate. Long-term transient myocardial ischemic attacks were related to particulates, and short-term ischemic attacks were related to nitrogen dioxide. CONCLUSIONS: Symptoms of ischemic cardiac and cerebrovascular diseases may be provoked by pollutants in concentrations lower than those given as guidelines in many countries and lower than previously shown.	
Objective: This study investigated whether low concentrations of ambient air pollutants are associated with hospital admissions for ischemic cardiac and cerebrovascular diseases.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean: 19±12.6 µg/m^3 Range: 0.2-95µg/m^3	Study period: 1096 days	No info	No info	N=12664 admissions due to ischemic cardiac diseases N=7232 admissions due to cerebrovascular episodes	No info	n/a	n/a

Observations:

There were no significant associations observed for SO_2 and health effects.
Sulfur dioxide had a highly significant negative correlation with temperature. Sulfur dioxide correlated at highly significant levels with nitrogen dioxide and total suspended particulates and correlated negatively with ozone.
Admissions due to all ischemic cardiac diseases showed a statistically significant association with pollutants in a model including all five pollutants. Overall

admissions due to cerebrovascular diseases did not show a significant association with pollution; however, admissions via the emergency room did show a significant association.

When the patients were analyzed separately in two age groups, the overall and emergency admissions due to all ischemic cardiac diseases were significantly associated with pollutants only among the younger patients.

Author's Conclusions:

There were no specific conclusions regarding associates between SO_2 and admissions.

"Our results suggest that even low-level air pollution may significantly exacerbate symptoms of ischemic cardiac and cerebrovascular diseases as measured by frequency of hospitalization. This observation is in agreement with a number of previous studies that have shown an association between mortality from these diseases and ambient air pollution and with recent studies suggesting that hospital admissions due to heart disease are also associated with air pollution. Our findings further suggest that these exacerbations may be associated with NO_2, which is derived from exhaust gases of car traffic, and not merely with particulates alone or with SO_2."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(more info needed)** Is the number of subjects sufficient to produce statistically significant results? **(-)** Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(+)** Were identical 'criteria for exclusion' applied to both cases and controls? **(+)**Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	**(+) Fixed Monitors** Was the exposure concentration measured? **(+) Study period 1987-1989**Is the exposure duration known?
C. Equipment:	**(-)** Was a full description of the monitoring devices provided? **(not given)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(-)** Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a on controls)** Were appropriate controls recruited? **(-)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(+)**Was the timing and length of follow-up appropriate? **(+) hospital admissions** Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? **(more info needed)** Were appropriate endpoints examined? **(-) no symptom data**Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity,

	onset and dose-response relationship provided for each symptom) (more info needed) Were accepted assessment methods employed? (more info needed) Were assessment measures sufficiently sensitive? (more info needed)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (more info needed) Did the investigator appropriately interpret the statistics? (more info needed) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (No specific conclusions re SO_2 made. Do the conclusions make biological sense? (No extrapolations made) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no significant or nonsignficant associations between cardiac and cerebrovascular diseases and SO_2 levels reported by the authors.

This study was novel in that it explored the effects of air pollution on the exacerbations of disease symptomatology. While results were in accord with previous findings of association between pollutants and mortality due to cardiovascular disease, the author concedes that relations may have been due to chance. There were also possible biases, such as the difference in basic population from day to day. Further studies need to be done to iron out any inconsistencies between the effects of various pollutants and hospital admissions due to ischemic cardiac and cerebrovascular diseases.

Confidence index:

There were four SO_2 monitoring stations and a map showing locations. However, there is no information on the variability between stations.

There was good detail on how cases were diagnosed.

More information is needed explaining some of the variables in the tables.

The regression results for SO_2 are not provided in the paper.

Most of the analysis groups all of the pollutants together. The details of the SO_2 analysis are not provided

This study attempted to control climatic parameters in its statistical analysis including temperature and relatively humidity, which were monitored hourly. Sites of monitoring stations were selected to attempt to represent the actual exposure of the population to ambient air pollutants.

This report suggests that during the study, SO_2 levels were lower than guideline levels, and as such, very few conclusions were made from SO_2 observations. Comments were made on pollution in general.

Review – Confidence index ranking:

Low to moderate

DRAFT

CASE-CONTROL REVIEW FORM

Author: Kelsall, J.E., Samet, J.M., Zeger, S.L. , Xu, J. **Internal ID:** 389

Title: Air Pollution and Mortality in Philadelphia, 1974-1988.

Year: 1997

Abstract: Analyses involving data from many locations throughout the world have now been conducted to assess the association between air pollution and mortality. To date, six independent analyses of mortality data for Philadelphia, Pennsylvania, have been reported. In this new analysis of Philadelphia data for 1974-1988, Poisson regression models were developed to estimate the increased risk of daily mortality associated with air pollution while controlling for longer-term time trends and season and for weather. Model development was based on prior understanding of the effects of these factors on mortality and on consideration of model fit. The authors found moderate correlations of daily concentrations of total suspended particles (TSP), sulfur dioxide (SO_2), nitrogen dioxide (NO2), and carbon monoxide (CO), and only slight correlations of ozone (O_3) with other pollutants. When included individually in the model, the means of current and previous days' levels of TSP, SO_2, and O_3 had statistically significant effects on total mortality; pollutant increases of an interquartile range (34.5 micrograms/m^3, 12.9 ppb, and 20.2 ppb, respectively) were associated with increases in mortality of around 1% for TSP and SO_2, and of around 2% for O_3. The effects of TSP and SO_2 were diminished when both pollutants were simultaneously included in the model, whether pairwise or in the full multi-pollutant model. These analyses confirm the association between TSP and mortality found in previous studies in Philadelphia and show that the association is robust to consideration of other pollutants in the model.

PIP: Data from various studies on the association between air pollution and mortality in Philadelphia, Pennsylvania, undertaken in the period 1974-1988 are used in a new analysis in which the authors attempt to control for the effects of temporal factors, weather, and other pollutants in order to estimate the independent effect of total suspended particles (TSP) on mortality. The results confirm the association between TSP and mortality found in previous studies in Philadelphia and show that the association is robust to consideration of other pollutants.

Objective: In the present analysis, we attempted to control for the effects of temporal factors, weather, and other pollutants in order to estimate the independent effect of TSP on mortality.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean 17.3±11.6ppb Range: 0.3-104.3 ppb	Study Period: 1974-1988	No info	All ages	N=daily counts of mortality	No info	n/a	n/a

Observations:

When SO_2 was included independently in the statistical model, the estimated percent change in mortality associated with an increase of one interquartile range (approximately 12.9 ppb) was 1.08 (95% CI 0.37-1.78).
There was little evidence of different pollutant effects by season.

There was no evidence of modification of pollutant effects by age.
The estimated percent change in mortality associated with an increase of one interquartile range (approx. 12.9 ppb) of SO_2 in the final model for each cause of death group were as follows: For cardiovascular disease 1.55 (95% CI 0.13-2.97), for respiratory disease 0.22 (-3.90-4.34) and for other causes 0.78 (95% CI -0.54-0.78).

Author's Conclusions:

There were no specific conclusions regarding SO_2 alone.

"We caution against using the model coefficients from this analysis directly to estimate the potential consequences of lowering concentrations of the individual pollutants through regulatory measures; the pollutant concentrations are correlated and the estimates of their effects depend on modeling assumptions."

"Given the strong association between TSP and SO_2, it is not possible, based solely on these Philadelphia data, to identify one or the other pollutant as the principal cause of increased mortality. These new models confirm that air pollution is associated with increased mortality in Philadelphia. This general conclusion has proved robust, and has been repeated by different groups of investigators using diverse analytic approaches, and applied to data from Philadelphia and from other locations."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitorsWas the exposure concentration measured? (+) Study period 1974-1988 Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a not applicable for this study) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc.) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc.)

E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) **mortality** Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? **(+/-) possible miscoding** Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable? (+)Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(No specific SO_2 conclusions)** Do the conclusions make biological sense? **(No extrapolations made)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The objective of this study was not to study the independent effects of SO_2 but rather that of TSP. However, results for SO_2 were given. The authors found moderate correlations of daily concentrations of sulfur dioxide (SO_2) with other pollutants. When included individually in the model, the means of current and previous days' levels of SO_2 non-statistically significant effects on total mortality; pollutant increases of an interquartile range 12.9 ppb) were associated with increases in mortality of around 1% for SO_2.

Future investigation is needed into whether the short-term associations represent the times of death of highly susceptible persons advancing by a few days, or an effect on a longer time frame.
This study found a strong correlation between TSP and SO_2. That said, it is not possible to identify which one of these pollutants is the principal cause of increased mortality.

Confidence index:
The main objective of the study was to look at effects of TSP, not SO_2. However, the study did provide useful information on how to handle different confounding variables such as weather, season and age. The authors gave a very detailed discussion comparing their results to other studies of Philadelphia air pollution effects. They made note of all of the reasons why making comparisons between the studies is difficult, such as measurement error, different analytical models, different time periods and different statistical models. They give information on studies that have shown that the strength of association will change depending on the statistical model used.
Though the authors do report some results for SO_2 and mortality, the results should be interpreted with caution as this was not the main objective. Also given the strong association between TSP and SO_2 it is not possible based solely on these Philadelphia data, to identify one or the other pollutant as the principal cause of increased mortality.
This is a good study. Results from the data analysis were presented very well graphically. Confidence intervals are included; however there is no individual or raw data and information on measurement equipment is missing. However, relatively speaking, this is not a big issue as other studies have not included them either.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Rahlenbeck, S.I., Kahl,H.	**Internal ID:** 391

Title: Air Pollution and Mortality in East Berlin during the Winters of 1981-1989

Year: 1996

Abstract: BACKGROUND: The relationship between air pollution and mortality in East Berlin was examined for the winters of 1981-1989. METHODS: Regression analysis included daily mean levels of sulphur dioxide (SO_2) and suspended particulates (SP), and was controlled for temperature, humidity, weekday, month, and year. Moving averages of previous pollution were also used. RESULTS: Each pollutant was a significant contributor to excess mortality. The strongest association was found for mortality lagged for 2 days, which depended significantly on the level of SP (beta for in SP = 0.876; P = 0.008) and SO_2 (beta for in SO_2 = 0.635; P = 0.012), when regressed separately. When omitting days with pollutant concentrations above 150 micrograms m^{-3}, the pollutant-mortality relationship was linear, and a 100 micrograms m^{-3} increase was associated with a 6.1% (SP) and 4.5% (SO_2) mortality increase 2 days later, when pollutants were considered separately; this was reduced to 4.6% (SP) and 2.8% (SO_2) increase, when both were considered simultaneously. CONCLUSIONS: The results show that short-term associations between air pollutants and mortality in East Berlin did exist during the winters 1981-1989. Since the coefficients for SP and SO_2 dropped when controlling for the other pollutant species, a similar strength of association with mortality for both pollutants was found.

Objective: In the present investigation, we examined the association between daily concentrations of SO_2 and SP and daily mortality in East Berlin, covering eight and a half winters, from Oct 1, 1981 to March 31, 1988 as well as from Oct 1, 1989 to Dec 31, 1989.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Range of means: 107-219 μg/m^3	Study period: 1981-1989 (winters)	**No info**	**No info**	**Population: 1,284,553 (1989)**	**No info**	**n/a**	**n/a**

Observations:

SO_2 was significantly associated with daily mortality in models using mortality lagged for 1, 2, and 4 days.
The effects estimates after omission of days with SO_2 levels > 150μg/m^3 , SO_2 was significantly associated with excess mortality, being associated with a 4.5% per 100 μg/m^3 increase in SO_2 levels.

Author's Conclusions:

"In summary, the association between air pollutants and daily mortality in East Berlin is quantitatively similar to results of other recent studies, though the often reported stronger mortality association of particulates compared to $SO_2$was not found here. The results show that short-term associations between air pollutants and mortality in East Berlin did exist during the winters 1981-1989. Since the coefficients for SP and SO_2 dropped when controlling for the other pollutant species, a similar strength of association with mortality for both pollutants was found."

> "Constraints of the present study include the imprecise exposure data derived from only one sampling point (the only one existing over the whole period), the lack of information on other pollutants existing, and finally, the use of a rather crude measure of health effects. Despite these constraints however, significant associations were found between SO_2 and SP levels and mortality in East Berlin. These associations were significant at concentrations well below air pollution standards."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **fixed monitors**Was the exposure concentration measured? (+) **study period** Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) **mortality counts from register** Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? **(n/a mortality counts)** Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) **mortality counts from register** Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a mortality counts)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not provided)** Did qualified personnel conduct the exams? (+) **all deaths occurring during study period** Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(n/a mortality counts)** Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?

F. Data analysis:	(-) more info needed Are the statistical methods used appropriate? (-) Are confidence intervals reported? (more info needed) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (+) some Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) (Do the conclusions make biological sense? No extrapolations madeAre the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Short-term associations between SO_2 and daily mortality (excluding deaths from accidents) in East Berlin were observed. However, problems in the study design make it difficult to ascertain whether the associations are real.

This study is novel in that it publishes data from East Berlin that was previously unavailable for evaluation. The author concedes that constraints of the present study include imprecise exposure data derived from only one sampling point. A significant association was observed between SO_2 and mortality in Berlin.

Confidence index:

There was only one monitoring station for SO_2.

The use of all-cause mortality may have led to spurious correlations.

There was extensive data manipulation and multiple models that made it difficult to know if the results were real.

There was also very little detail given on the statistical models used.

There were no relative risks, odds ratios or confidence intervals reported.

Some of the variables in the table are not clearly explained.

The use of dummy variables in some of the models may have led to over specification or under specification. There is little detail given. It seems that the data was manipulated until a significant association occurred.

This study attempted to limit confounding factors by controlling for weather variables using multiple regression models. Models were also controlled for covariates by use of moving averages. While this study produced significant findings, confidence intervals were not given.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Hwang, J.S., Chan, C.C.	**Internal ID:** 393

Title: Effects of Air Pollution on Daily Clinic Visits for Lower Respiratory Tract Illness

Year: 2002

Abstract: The authors used data obtained from clinic records and environmental monitoring stations in Taiwan during 1998 to estimate the association between air pollution and daily numbers of clinic visits for lower respiratory tract illness. A small-area design and hierarchical modeling were used for the analysis. Rates of daily clinic visits were associated with current-day concentrations of nitrogen dioxide, carbon monoxide, sulfur dioxide, and particulate matter less than or equal to 10 microm in aerometric diameter. People over age 65 years were the most susceptible, and estimated pollution effects decreased as the exposure time lag increased. The analysis also suggested that several community-specific variables, such as a community's population density and yearly air pollution levels, modified the effects of air pollution. In this paper, the authors demonstrate the use of a small-area design to assess acute health effects of air pollution.

Objective: In this study, we used a new design for clustering data and a Bayesian hierarchical modeling strategy to estimate effects of air pollution on daily clinic visits for lower respiratory tract illness across small study areas in Taiwan.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Mean: 5.4±3.0 ppb Range: 1.5-16.9 ppb	Study period: 1998	No info	All ages	For the 50 communities: Population at risk: 19,000-278,000	No info	n/a	n/a

Observations:

The overall effects of pollution on clinic visits due to lower respiratory tract illness were calculated by using national average concentrations as baseline exposures and percentages above the national average level as contrast exposures in the hierarchical models.
Significant heterogeneity for environmental data and outcomes among the 50 study communities were observed.
The variation in clinic visits was statistically significantly related to variation in SO_2 exposures.
The results of the single pollutant models for an all-ages population with current-day exposure showed that overall clinic visits were significantly related to SO_2exposures.
The pollution effects were always greatest for current-day exposures and decreased significantly as exposure time lags increased for sulfur dioxide.
People over age 65 years were the most susceptible.
The analysis also suggested that several community-specific variables, such as a community's population density and yearly air pollution levels, modified the effects of air pollution.

Author's Conclusions:

"Bayesian hierarchical models with community-specific covariates can smooth out heterogeneous estimates but still retain meaningful information on variations in pollution effects among communities. Accordingly, through a small area design and Bayesian hierarchical models, one can efficiently obtain national risk estimates for air pollution effects and improve community specific estimates or individual communities. We believe that such a design modeling approach could be used more widely in environmental epidemiologic studies."

"Our study found that nitrogen dioxide, carbon monoxide, sulfur dioxide, and PM_{10} all had significant estimated effects on daily clinic visits due to lower respiratory tract illness in Taiwan.

Chronic PM_{10} exposures slightly modified the estimated acute exposure effects of nitrogen dioxide and sulfur dioxide, even after each community's population density and temperature were adjusted for in the models. These findings indicate that in the future we must consider multiple pollutants and interactions between acute and chronic exposures simultaneously in order to estimate individual air pollutants' concentration-response relations."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **fixed monitors**Was the exposure concentration measured? (+) **1 year study period** Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+) **hospital admission data**Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) **ICD-9 codes**Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive?

	(-) Was the nature and severity of symptoms graded against an explicitly defined scale? (info not provided) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+/-)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (unclear) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+/-)Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a no conclusions re SO_2) Do the conclusions make biological sense? (no extrapolations re SO_2 made) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The authors used data obtained from clinic records and environmental monitoring stations in Taiwan during 1998 to estimate the association between air pollution and daily numbers of clinic visits for lower respiratory tract illness. Rates of daily clinic visits were associated with current-day concentrations of sulfur dioxide, nitrogen dioxide, carbon monoxide, and PM_{10}. People over age 65 years were the most susceptible.

The estimated effects of air pollutants appeared to decrease as exposure time lags increased. Future studies should explore the biological plausibility of respiratory illnesses being caused or exacerbated by specific air pollutants.

This study suggests that several community-specific variables, such as a community's population density and yearly air pollution levels, modified the effects of air pollution.

Confidence index:

Because this study used different data analysis methods than most other studies, it is difficult to compare the results.

The population at risk had to be estimated under the assumption of stability across time. The population at risk was estimated from the medical claims data of small private clinics and medium-sized medical institutions in Taiwan's National Health Insurance Program.

The models relied on the inclusion of many dummy variables which may have affected the results.

Only the detailed results for NO_2 were shown in the paper.

The analysis also suggested that several community-specific variables, such as a community's population density and yearly air pollution levels, modified the

effects of air pollution.
The analysis for statistical significance was unclear.
This study attempted to limit confounding factors by classifying the estimate population into three groups: children, adults and the elderly. Furthermore, meteorological measurements were considered as were daily health records.
This study's weakness is in exposure assessment; however, there is discussion of the potential limitations on interpretation based on this weakness.
Confidence intervals are not reported.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Lin, M., Chen, Y., Burnett, R.T., Villeneuve, P.J., Krewski, D.	**Internal ID:** 394

Title: Effect of short-term exposure to gaseous pollution on asthma hospitalization in children: a bidirectional case-crossover analysis

Year: 2003

Abstract: STUDY OBJECTIVE: Assess associations between short-term exposure to gaseous pollutants and asthma hospitalization among boys and girls 6 to12 years of age. DESIGN: A bi-directional case-crossover analysis was used. Conditional logistic regression models were fitted to the data for boys and girls separately. Exposures averaged over periods ranging from one to seven days were used to assess the effects of gaseous pollutants on asthma hospitalization. Estimated relative risks for asthma hospitalization were calculated for an incremental exposure corresponding to the interquartile range in pollutant levels, adjusted for daily weather conditions and concomitant exposure to particulate matter. SETTING: Toronto, Ontario, Canada. PARTICIPANTS: A total of 7319 asthma hospitalizations for children 6 to 12 years of age (4629 for boys and 2690 for girls) in Toronto between 1981 and 1993. MAIN RESULTS: A significant acute effect of carbon monoxide on asthma hospitalization was found in boys, and sulphur dioxide showed significant effects of prolonged exposure in girls. Nitrogen dioxide was positively associated with asthma admissions in both sexes. The lag time for certain gaseous pollutant effects seemed to be shorter in boys (around two to three days for carbon monoxide and nitrogen dioxide), as compared with girls (about six to seven days for sulphur dioxide and nitrogen dioxide). The effects of gaseous pollutants on asthma hospitalization remained after adjustment of particulate matter. The data showed no association between ozone and asthma hospitalization in children. CONCLUSIONS: The study showed positive relations between gaseous pollutants (carbon monoxide, sulphur dioxide, and nitrogen dioxide) at comparatively low levels and asthma hospitalization in children, using bi-directional case-crossover analyses. Though, the effects of certain specific gaseous pollutants were found to vary in boys and girls.

Objective: Assess associations between short-term exposure to gaseous pollutants and asthma hospitalization among boys and girls 6 to12 years of age.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Mean: $SO_2$5.36 ppb	Study period: 1981-1993	Boys=4629 hospitalizations Girls=2690 hospitalizations	6-12 years old	7319 asthma hospitalizations	No info	n/a	n/a

Observations:

SO_2showed significant effects of prolonged exposure in girls.

For SO_2, (SO_2 = 7 ppb) only six and seven day averaging exposures showed a significant effect in girls; these effects were not apparent in boys.

The effects of gaseous pollutants on asthma hospitalization remained after adjustment of particulate matter.

Correlations between gaseous and particulate air pollutants and weather conditions were calculated. Gaseous pollutants were all positively correlated with each other, with the exception of ozone. The gaseous pollutants were all positively correlated with ambient particulate matter.

Author's Conclusions:

The study showed positive relations between gaseous pollutants (carbon monoxide, sulphur dioxide, and nitrogen dioxide) at comparatively low levels and asthma hospitalization in children, using bi-directional case-crossover analyses. Throughout, the effects of certain specific gaseous pollutants were found to vary in boys and girls.

"Six and seven day averaging exposures to SO_2 showed a significant effect on asthma hospitalization in girls, but not in boys in this study. Some human controlled studies have found that asthmatic children are more sensitive to SO_2 than healthy subjects, with SO_2 concentrations below 250 ppb resulting in temporary breathing impairment and reduced lung function. Most previous epidemiological studies have not demonstrated a significant effect of SO_2 on asthma hospitalization or emergency room visits. However, SO_2 exposures of 80 ppb or less were found to be related to asthma hospitalization in some studies. There are several possible explanations for these inconsistent results. Firstly, as SO_2 can only reach the gas exchange region of the lungs after sorption into fine particles, SO_2 effects may not be observed at high concentrations. Secondly most previous studies did not examine the prolonged exposure effects of SO_2. And thirdly, the effects of SO_2 seem to be age and gender related, which have not been well studied in previous studies."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **fixed monitors**Was the exposure concentration measured? (+) **study period 1981-1993** Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? **(more info needed)** Were appropriate endpoints examined? **(more info needed)** Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(more info needed)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions?

	(-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (more info needed) Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (-) Do the conclusions make biological sense? (no extrapolations made) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Using the bi-directional case-crossover design, this study found SO_2 to be positively associated with asthma hospitalization, although the relations varied in boys and in girls. In girls, sulphur dioxide showed significant effects of prolonged exposure, while no effect was seen in boys. However, the significant relative risks the authors refer to were close to null and therefore further evidence is needed to study a possible gender difference.

Confidence index:

The hospital admissions due to asthma represent extreme cases only and therefore it is difficult to generalize to the whole populations.

The study design was a bi-directional crossover analysis, which made it difficult to compare to other studies.

The control period for the bidrectional crossover design was chosen to be two weeks before and after the admission date. The length of the control period may have an effect on the results.

There was no information on the number of monitors, location, techniques etc.

This study uses children between the ages of 6-12. Use of this age range is explained based on children being more susceptible to air pollution than adults under 65 years. In addition, this study used periods of control. Conditional logistic regression methods were used.

This study was weak in exposure assessment and reporting. No raw data or individual data was reported.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Burnett, R.T., Cakmak, S., Brook, J.R.	**Internal ID:** 395

Title: The Effect of the Urban Ambient Air Pollution Mix on Daily Mortality Rates in 11 Canadian Cities

Year: 1998

Abstract: OBJECTIVE: Determine the risk of premature mortality due to the urban ambient air pollution mix in Canada. METHODS: The number of daily deaths for non-accidental causes were obtained in 11 cities from 1980 to 1991 and linked to concentrations of ambient gaseous air pollutants using relative risk regression models for longitudinal count data. RESULTS: Nitrogen dioxide had the largest effect on mortality with a 4.1% increased risk ($p < 0.01$), followed by ozone at 1.8% ($p < 0.01$), sulphur dioxide at 1.4% ($p < 0.01$), and carbon monoxide at 0.9% ($p = 0.04$) in multiple pollutant regression models. A 0.4% reduction in premature mortality was attributed to achieving a sulphur content of gasoline of 30 ppm in five Canadian cities, a risk reduction 12 times greater than previously reported. CONCLUSIONS: Ambient air pollution generated from the burning of fossil fuels is a risk factor for premature mortality in 11 Canadian cities.

Objective: The purpose of the present investigation was to determine the risk of premature mortality due to exposure to mixtures of gaseous ambient air pollutants in 11 Canadian Cities.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Daily Average SO_2 for 11 Canadian Cities: 0.7-10.5 ppb Daily Average for all cities combined: 5.4ppb	Study period: 1980-1991	No info	No info	816,991 deaths for nonaccidental causes between 1980 – 1991 comprising a population of 10.8 million people	No info	n/a	n/a

Observations:

The percentage increased risk of death attributable to a change in study mean SO_2 concentrations separately by city, varied from 0.1% to 4.2%. The percentage increase for all of the cities combined was 1.8%.

The rest of the observations looked at the effects of SO_2 combined with all of the other pollutants: The combined percentage increased risk of death attributable to change in study mean SO_2 concentrations examined simultaneously by city was 1.4%.

Nitrogen dioxide had the largest effect on mortality with a 4.1% increased risk ($p<0.01$), followed by ozone at 1.8% ($p<0.01$), SO_2 at 1.4% ($p<0.01$), and carbon

monoxide at 0.9% (p=0.04) in multiple pollutant regression models.
A 0.4% reduction in premature mortality was attributed to achieving a sulfur content of gasoline of 30 ppm in five Canadian cities, a risk reduction 12 times greater than previously reported.

Author's Conclusions:

Ambient air pollution generated from the burning of fossil fuels is a risk factor for premature mortality in 11 Canadian cities.
"The risks associated with sulfur dioxide exposure are greater in those cities with lower $PM_{2.5}$. Thus $PM_{2.5}$ may act as a potential confounder for these latter two pollutants (SO2 and O3). There is no apparent association, however, between the total risk of the air pollution mix, based on multiple pollutant models and $PM_{2.5}$ concentrations, suggesting that the four gaseous pollutants can adequately explain daily variations in mortality rates and that the addition of $PM_{2.5}$ is unlikely to add any additional predictive power."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed montironsWas the exposure concentration measured? (+) study period 1980 to 1991 Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (not provided) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom)

	(+) **mortality counts**Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a mortality counts)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) **mortality counts**Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? **(more info needed)** Did the investigator appropriately interpret the statistics? **(more info needed)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

When the four pollutants were examined simultaneously, the average risk for sulphur dioxide was 1.4% $p<0.01$. However, the relative risks varied among the cities with little consistency within regions of Canada.

The study indicated that risks associated with SO_2 exposure may be higher in conjunction with $PM_{2.5}$ levels.

This study points out that ambient air pollution is associated with premature mortality in 11 Canadian cities. It was found that while air pollution mitigation strategies have been designed to reduce ambient levels of $PM_{2.5}$ and PM_{10} levels of several gaseous pollutants were also predicted to be reduced, thereby indicating an association between PM and SO_2.

Confidence index:

Confidence intervals were not reported.

There is no information on the monitoring systems in each city. Some of the concentrations of SO_2 were quite low; however, there is no information given re. the sensitivity of the monitoring instruments.
The relative risks varied considerably among the cities.
The main focus of the study was the mixture of pollutants, not SO_2 separately.
Some of the variables in the tables needed more explanation.
Methods section needed more detail, particularly re. the statistical methods.
This study made use of an extremely large population. Description of the demographics or other characteristics of the population was not recorded. The study employed city-specific long-term trends, seasonal and sub-seasonal cycles using non-parametric smoothed functions.

Review – Confidence index ranking:

Low to moderate

DRAFT

CASE-CONTROL REVIEW FORM

Author: Hong, Y.C., Lee, J.T., Kim, H., Kwon, H.J. **Internal ID:** 397

Title: Air Pollution: A New Risk Factor In Ischemic Stroke Mortality

Year: 2002

Abstract: BACKGROUND AND PURPOSE: Air pollution is known to be associated with cardiovascular disease, but little is known about the occurrence of stroke in relation to air pollution. We investigated the association between acute stroke mortality and air pollution over a 7-year period (January 1991 through December 1997) in Seoul, Korea. METHODS: A generalized additive model was used to regress daily stroke death counts for each air pollutant, controlling for time trends, day of the week, and meteorological influences such as temperature, relative humidity, and atmospheric pressure. Ischemic and hemorrhagic stroke deaths were examined separately. RESULTS: The effects of air pollutants on ischemic stroke mortality were statistically significant, whereas this was not the case for hemorrhagic stroke mortality. We observed estimated relative risks of 1.03 (95% CI, 1.00 to 1.06) and 1.04 (95% CI, 1.01 to 1.08) for ischemic stroke mortality for each interquartile range increase in total suspended particulates and sulfur dioxide concentrations on the same day. We also found significantly increased relative risks of 1.04 (95% CI, 1.01 to 1.07) for nitrogen dioxide with a 1-day lag, of 1.06 (95% CI, 1.02 to 1.09) for carbon monoxide with a 1-day lag, and of 1.06 (95% CI, 1.02 to 1.10) for ozone with a 3-day lag for each interquartile range increase. CONCLUSIONS: These findings indicate that air pollutants are significantly associated with ischemic stroke mortality, which suggests an acute pathogenetic process in the cerebrovascular system induced by air pollution.

Objective: We hypothesize that air pollutants may have significant effects on mortality through acute stroke events. In this analysis, we examined the association between air pollutants and ischemic and hemorrhagic stroke mortality.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean Values 21.8±18.8ppb	Jan 1991-Dec 1997	No info	No info	Mortality : approx. 7.4 deaths/day from stroke	No info	Na/	Na/

Observations:

The estimated relative risks of ischemic stroke mortality for each interquartile range increase in SO_2 (17.43ppb) was significant at RR=1.04 (95% CI 1.01-1.07).
The results for hemorrhagic stroke mortality were not significant.
Relative risk of 1.03 for ischemic stroke mortality was observed for each interquartile range increase in total suspended on the same day.
The Poisson regression model included time trends, day of the week, and weather variables. To determine the lag effects of air pollutants on stroke mortality, the relative risks of different lag models, with a maximum lag of days were compared. The associations between TSP and SO2 and ischemic stroke mortality were highest on the same day.

Author's Conclusions:

"In summary, air pollution was significantly associated with ischemic stroke mortality in Seoul. These findings support the possibility that acute pathogenetic processes in the cerebrovascular system could be induced by the air pollution."

"The increase of relative risk of dying from ischemic stroke with increased air pollution is on the order of 3% to 6%, depending on the air pollutant being investigated. This relative risk increase is potentially important, given that the mortality of ischemic stroke is 2.8 per day in this city, which indicates that 214 to 428 ischemic stroke deaths were due to air pollution in the study period. It is noteworthy that gaseous pollutants showed robust associations with ischemic stroke mortality in the 2-pollutant models. However, TSP may not work well as a surrogate for fine particles, which are believed to be a risk factor of premature mortality. Because fine particles can form as a result of chemical reactions between gases in the atmosphere and because these particles dominate the inhalable particulate fraction, gaseous pollutants such as SO_2 and NO_2 may be good markers for such particles."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(more info needed)** Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (+)Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(n/a)** Was the timing and length of follow-up appropriate? **(n/a)** as the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) **for the objective**Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions?

	(-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (-) Do the conclusions make biological sense? **(no extraps)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)**Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

A statistically significant association was observed between $SO_2$and ischemic stroke mortality. The RR was 1.04 and the 95% CI was 1.01-1.08. These associations were observed after taking into account time trends, day of the week, and meteorological variables.

Very little is known about the association between air pollution and stroke, particularly the differences between ischemic and hemorrhagic stroke in terms of their association with air pollution. The findings from this study support the possibility that acute pathogenetic processes in the cerebrovascular system could be induced by the air pollution.

The findings need to be confirmed by additional studies on stroke mortality and SO_2.

Confidence index:

There is no detail on the distribution of the monitoring locations and the measurements of SO_2.

The confidence interval for the RR was relatively wide.

There is the possibility in misscoding for the type of stroke.

Similar to most other environmental epidemiology studies on air pollution, this study was limited in that it used ambient environmental monitoring data as exposure data. These data do not necessarily represent individual exposures. However, another measurement error resulting from a difference between the measured and true ambient levels was minimized or circumvented by the use of data from 20 air-monitoring stations evenly located within the study area. The author also points out that individual risk factors and daily variations in these risk factors most likely do not correlate with daily changes in air pollution; therefore, they were most likely not confounding factors in the time-series study.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Lee, J.T., Kim, H., Song, H., Hong, Y.C., Cho, Y.S., Shin, S.Y., Hyun, Y.J., Kim, Y.S.	**Internal ID:** 398

Title: Air Pollution and Asthma Among Children in Seoul, Korea

Year: 2002

Abstract: BACKGROUND: As information about the health risks associated with air pollution has become available, attention has focused increasingly on susceptible persons such as children and persons with preexisting respiratory diseases, such as asthma. METHODS: We investigated the association between outdoor air pollution and asthma attacks among children under 15 years of age in Seoul, Korea. We estimated the relative risks of hospitalization associated with an interquartile range (IQR) increase in pollutant concentrations and used time series analysis of the counts by means of the generalized additive Poisson model. RESULTS: The estimated relative risk of hospitalization for asthma was 1.07 (95% confidence interval [CI] = 1.04-1.11) for particulate matters less than or equal to 10 microm in aerodynamic diameter (IQR = 40.4 microg/m^3); 1.11 (95% CI = 1.06-1.17) for sulfur dioxide (IQR = 4.4 ppb); 1.15 (95% CI = 1.10-1.20) for nitrogen dioxide (IQR = 14.6 ppb); 1.12 (95% CI = 1.07-1.16) for ozone (IQR = 21.7 ppb); and 1.16 (95% CI = 1.10-1.22) for carbon monoxide (IQR = 1.0 ppm). CONCLUSIONS: These findings support the hypothesis that air pollution at levels below the current standards of Korea is harmful to sensitive subjects such as asthmatic children.

Objective: Our objective was to estimate the association between ambient air pollution and hospital admissions for asthma among children 14 years of age or younger.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean SO_2 : 7.7$\pm$3.3ppb Interquartile Range: 4.4 ppb	Study period: 2 years	Male and female	<15 yrs	6436 asthma-related hospital admissions	No info	n/a	n/a

Observations:

The estimated relative risk of hospital admissions for asthma was 1.07 (95% confidence interval [CI] = 1.04-1.11) for particulate matters less than or equal to 10 μm in aerodynamic diameter (IQR = 40.4 μg/m^3); 1.11 (95% CI = 1.06-1.17) for sulfur dioxide (IQR = 4.4ppb); 1.15 (95% CI = 1.10-1.20) for nitrogen dioxide (IQR = 14.6 ppb); 1.12 (95% CI = 1.07-1.16) for ozone (IQR = 21.7 pbb); and 1.16 (95% CI = 1.10-1.22) for carbon monoxide (IQR = 1.0 ppm).
In the multipollutant model for correlation analysis of the air pollutants, colinearity between NO_2 and PM_{10}, and SO_2 and CO could explain the changes in the estimates of the other pollutant in the two-pollutant models when NO_2 was introduced.

Author's Conclusions:

"This study reinforces the possible role of particles and SO_2 on asthma attacks, although it should be interpreted with caution because the relative risks are close to the null and because the results in the multipollutant models are consistent. Children with asthma appear to be a population at particular risk to the inhaled effects of air pollution at concentrations that commonly occur in most countries."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitorsWas the exposure concentration measured? (+) study period 2 yearsIs the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (No info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (more info needed) Were accepted assessment methods employed? (more info needed) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (not stated) Are the observed trends statistically significant?

G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (more info needed) Were assumptions reasonable? (-) Are the methods reproducible? some Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The estimated relative risk of hospitalization for asthma was 1.11 (95% CI = 1.06-1.17) for SO_2 . These results may indicate that SO_2 levels below current standards of Korea are harmful to asthmatic children; however, they should be interpreted with caution because the relative risks are close to the null and because the results in some of the models were inconsistent.

A proposed mechanism by which air pollutants exert their health effects involves the production of a large amount of reactive oxygen species in the respiratory tracts by some exogenous oxidants such as air pollutants.

Confidence index:

Possible misdiagnosis in very young children.

Confounders such as influenza not considered.

Very limited information on monitoring stations and sensitivity.

The results of the multipollutant models were inconsistent.

This study attempted to record the effects of multiple pollutants on a sample group. The size of the sample group was appropriate to extrapolate results to the broader population. Furthermore, since the investigation did not target subjects with specific attributes (other than asthma), it is likely that the subject group reflects the larger population of children in Seoul.

Exposure assessment is weak.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Martins, L.C., Latoree, M.R.D.O., Saldiva, P.H.N., Braga, A.L.F.B.	**Internal ID:** 399

Title: Air Pollution and Emergency Room Visits Due to Chronic Lower Respiratory Diseases in the Elderly: An Ecological Time Series Study in Sao Paulo, Brazil

Year: 2002

Abstract: The objective of this study was to investigate the effect of daily air pollution levels (carbon monoxide, nitrogen dioxide, ozone, sulfur dioxide, and particulate matter with an aerodynamic profile < or = 10 microns) on morbidity by using the daily number of emergency room visits due to chronic lower respiratory disease (CLRD) in people older than 64 years of age in the city of Sao Paulo, Brazil, from 1996 to 1998. Generalized additive Poisson regression models adjusted for long-time trend and weather (nonparametric functions), weekdays (dummy variable), and daily number of nonrespiratory admissions were adopted. Ozone and sulfur dioxide were the pollutants statistically associated with CLRD visits. Interquartile range increases in the 6-day moving average of sulfur dioxide (11.82 micrograms/m^3) and in the 4-day moving average of ozone(35.87 micrograms/m^3) increased CLRD emergency room visits in 18% and 14%, respectively. These results reinforce the idea that air pollution may promote adverse health effects in the elderly.

Objective: The objective of this study was to investigate the effect of daily air pollution levels (carbon monoxide, nitrogen dioxide, ozone, sulfur dioxide, and particulate matter with an aerodynamic profile < or = 10 microns) on morbidity by using the daily number of emergency room visits due to chronic lower respiratory disease (CLRD) in people older than 64 years of age in the city of Sao Paulo, Brazil, from 1996 to 1998.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean SO_2: 18.7 ± 10.6 $\mu g/m^3$	Study Period: 1996-1998	Male and female	>64y	13,163 elderly emergency room attendances 2300 due to respiratory disease 712 due to CLRDs	Chronic lower respiratory disease	n/a	n/a

Observations:

SO_2 (6 day moving average) was significantly and positively associated with ER visits due to chronic lower respiratory disease (CLRD).
A increase in the six-day moving average interquartile range for SO_2 (11.82 $\mu g/m^3$) increased the emergency room visits by 18% (Range 4.14% - 31.85%).

Interquartile range increases in the 4-day moving average of ozone (35.87 $\mu g/m^3$) increased CLRD emergency room visits by 14% respectively.
In the single-pollutant models, all pollutants were positively associated with CLRD visits, although only O_3 and SO_2 were statistically significantly associated with the outcome. When a co-pollutant model was used, the effect of O_3 remained virtually unchanged, whereas the effect of SO_2 decreased by about 26%.

Author's Conclusions:

"In conclusion, we have presented an association between SO_2 with CLRD emergency room visits in Sao Paulo, Brazil. This association was robust enough to resist the inclusion of the confounders usually used in air pollution adverse health effect time series studies. Also, we showed that an air pollutant can remain a serious public health problem even when its concentration is under the respective air quality standard."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitorsWas the exposure concentration measured? (+) 1996-1998Is the exposure duration known?
C. Equipment:	(+) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	((+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (+)Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+/-) possible misdiagnosis Were assessment measures sufficiently sensitive? (no info) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical?

	(some) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (+) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (more info needed)Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

SO_2 was significantly associated with CLRD admissions at levels below the World Health Guidelines. This suggests that older persons suffering from respiratory disease are more susceptible to low levels of SO_2.

This study suggests that SO_2 may affect healthy adults by increasing respiratory symptoms, respiratory mortality, and hospital visits by decreasing lung function.

Confidence index:

$SO_2$was measured at 13 stations. However there is no further information on the variability between stations, measurements, sensitivity, location, etc. Exposure measurement and classification is weak.

Provided justification on why the cases chosen were representative.

No mention of possible confounding by influenza.

The hospital used for the study receives people who look to the hospital as their first choice for medical care, as well as people who are directed from other medical centers. The proportion of patients that the hospital receives from other centers is unknown.

Small number of daily number of cases recorded at the hospital used.

Confidence intervals are fairly wide.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Ballester, F., Saez, M., Perez-Hoyos, S., Iniguez, C., Gandarillas, A., Tobias, A., Beilido, J., Taracido, M., Arribas, F., Daponte, A., Alonso, E., Canada, A., Guillen-Grima, F., Cirera, L., Perez-Boillos, M.J., Saurina, C., Gomez, F., Tenias, J.M.	**Internal ID:** 400

Title: The EMECAM project: a multicentre study on air pollution and morality in Spain: combined results for particulates and for sulfur dioxide.

Year: 2002

Abstract: OBJECTIVE: The EMECAM study is a collaborative effort to evaluate the impact of air pollution on mortality in Spain. In this paper the combined results are presented for the short term effects of particulates and sulfur dioxide on both daily mortality for all and for specific causes. METHODS: The relation between daily mortality for all causes, cardiovascular diseases, and respiratory diseases, and air pollution for particulates (daily concentrations) and SO_2 (24 and 1 hour concentrations) was assessed in 13 Spanish cities for the period 1990-6. With a standardised method, magnitude of association in each city was estimated by Poisson regression in a generalised additive model. Local estimates were obtained from both single and two pollutant analyses. Lastly, combined estimates for each cause and pollutant were obtained. RESULTS: For combined results, in single pollutant models a 10 microg/m^3 increase in the concentration of the mean of the concurrent and one day lag for black smoke was associated with a 0.8% (95% confidence interval (95% CI) 0.4 to 1.1%) increase in total mortality. The estimates for total suspended particles (TSPs) and particulate matter of aerodynamic diameter <10 microm (PM_{10}) and total mortality were slightly lower. The same increase in concentrations of SO_2 was associated with a 0.5% increase in daily deaths. For groups of specific causes, higher estimations were found, especially for respiratory conditions. Peak concentrations of SO_2 showed significant associations with the three groups of mortality. When two pollutant analyses were performed, estimates for particulates, especially for black smoke, did not substantially change. The estimates for daily concentrations of SO_2 were greatly reduced, but, on the contrary, the association with peak concentrations of SO_2 did not show any change. CONCLUSIONS: There is an association between mortality and pollution through particulates among city populations in Spain. Peak rather than daily concentrations of SO_2 were related to mortality. Results suggest that populations in Spanish cities are exposed to health risks derived from air pollution.

Objective: The EMECAM study is a collaborative effort to evaluate the impact of air pollution on mortality in Spain. In this paper the combined results are presented for the short term effects of particulates and sulfur dioxide on both daily mortality for all and for specific causes.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Daily mean SO_2: Range for 13 cities 8.1-44.5μg/m^3	Study period: 1990-1996	Male and female	No info	Mean total daily deaths: 2.5-60.9 Populations: 134000-2.9 million	No info	n/a	n/a

Observations:

When the cities were analyzed separately, for an increase in SO_2 of 10μg/m^3, the RRs were all very low and some of the 95% CI included RR=1.00 for total mortality, cardiovascular mortality and respiratory mortality.

Peak concentrations of SO_2 showed different relations with mortality than did daily concentrations.

Selected RRs

Summary RRs and 95% CI of mortality associated with a 10 $\mu g/m^3$ increase in SO_2 across the EMECAM cities (all causes excluding external) were not significant: for the 24h mean, lag 0 and lag 1, RR=1.005 (1.001-1.010) and for the 1h maximum, lag 0, 1.001 (1.000-1.003).

For cardiovascular mortality: 24 hour mean SO_2, lag 0 and lag 1, RR=1.005(0.998-1.011) and for the 1hour max., lag 0, RR=1.001 (0.999-1.003). These results were not significant. The results for respiratory mortality were similar and also not significant.

The authors used two different models to analyze the data, a random effects model and a fixed effects model. The results presented above are for the random effects model. The fixed effects model results were similar

For combined results, in single-pollutant models a 10 $\mu g/m^3$ increase in the concentration of the mean of the concurrent and one day lag for black smoke was associated with a 0.8% (95% confidence interval (95% CI) 0.4 to 1.1%) increase in total mortality. The estimates for total suspended particles (TSPs) and particulate matter of aerodynamic diameter < 10μm (PM_{10}) and total mortality were slightly lower. The same increase in concentrations of SO_2 was associated with a 0.5% increase in daily deaths. For groups of specific causes, higher estimations were found, especially for respiratory conditions. Peak concentrations of SO_2 showed significant associations with the three groups of mortality. When two-pollutant analyses were performed, estimates for particulates, especially for black smoke, did not substantially change. The estimates for daily concentrations of SO_2 were greatly reduced, but, on the contrary, the associations with peak concentrations of SO_2 did not show any change.

Author's Conclusions:

"To conclude, the results presented here show an association between mortality and pollution through particulates among city population in Spain, although a confounding effect from other pollutants cannot be excluded. This association is greater for groups of specific causes. The 1hr SO_2 maximum presents a clearer impact on mortality than the 24 hour SO_2, particularly for respiratory causes. However, results are not homogeneous among the cities. In some of them, there is a lack of results showing evidence of associations, or else these results are of little consistency."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (more info needed) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitorsWas the exposure concentration measured? (+) study period 7 yearsIs the exposure duration known?

C. Equipment:	(-) Was a full description of the monitoring devices provided? (No info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) **mortality counts** Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) **mortality** Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+/-) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) **mortality counts** Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (-) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable?

	(+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no significant associations between SO_2 and mortality (all causes, respiratory and cardiovascular). The authors reported one finding to be statistically significant (a 10µg/m^3 increase in SO_2 was significantly associated with a 0.2% excess in total deaths (fixed effects model) in both single and two pollutant models). However, it didn't appear that these results were significant. The corresponding RR was null.

Peak concentrations of SO_2 showed different relations with mortality than did daily concentrations.

Confidence index:

There was a lot of variability between the cities, including the geography, population and SO_2 levels.

Four of the cities used manual monitoring rather than automatic. The detection methods were different for each monitor.

The number of monitors changed depending on the city.

Two models were used for analysis. For the random effects model, the data from all 13 cities were used. For the fixed effects model, only 7 cities were used.

Because few studies were combined, the statistical power of the analysis is likely very small.

The pollutant concentrations had high standard deviations.

The period of study differed for each city.

The figures for RR were difficult to read.

Results reported as statistically significant, did not appear to be significant. See under Discussion of Findings.

This study notes that each city represents different social demographic, climatic and environmental situations. However, the author does not indicate specific locations of number of monitors used in the study, making it hard to reproduce.

This study evaluated 1hr SO_2 concentrations as well as 24hr SO_2 concentrations. An in-depth report on analysis and discussion was given which included multiple charts and graphs to help interpretation.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Desqueyroux, H., Pujet, J.C., Prosper, M., Squinazi, F., Momas, I.	**Internal ID:** 402
Title: Short-Term Effects of Low-Level Air Pollution on Respiratory Health of Adults Suffering from Moderate to Severe Asthma	
Year: 2002	
Abstract: Only a few studies have been carried out on the health effects of air pollution on patients suffering from severe asthma. We wanted to test the sensitivity of these patients to Paris air pollution. During 13 months, 60 severe asthmatics (62- female; mean age 55 years) were monitored by their physician, who filled in a follow-up form at each consultation and reported any asthma attacks. Daily levels of SO_2, PM_{10}, NO_2, and O_3 were provided by the air quality network. Statistical analysis (generalized estimating equation models that accounted for autocorrelation of responses, temporal, meteorological, and aerobiological variables, and some individual characteristics) revealed significant associations between PM_{10}, O_3, and incident asthma attacks. Odds Ratio (OR) for an increase of 10 microg/m^3 of PM_{10} was 1.41; 95% confidence interval (CI) [1.16; 1.71]. An increase of 10 microg/m^3 of O_3 was significantly associated with asthma attacks; OR=1.20; 95% CI [1.03; 1.41]. These relations were observed after a delay between exposure and asthma attacks of 3 to 5 days for PM_{10} and 2 days for O_3, and they tended to differ according to atopic status. The results of our study suggest that ambient Paris levels of PM_{10} and O_3 affected health of severe asthmatics, despite their treatment. 2002 Elsevier Science (USA)	
Objective: The aim of this study was to follow up a new panel of asthmatics over a 1-year period, to determine the short-term effects of winter air pollution (particles with an aerodynamic diameter less than 10μm; PM_{10}, SO_2 , and NO_2) and photochemical air pollution.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Summer Mean:7±5μg/m^3 Range:2-27μg/m^3 **Winter** Mean: 19±12μg/m^3 Range: 3-81μg/m^3	Study period: 1 year	23 male and 37 female	Mean age: 55 ±17 years	60	Severe asthmatic	n/a	n/a

Observations:

No association was shown between asthma attacks and SO_2 whatever the lag or season. The ORs for a 10μg/m^3 increase were all close to null and the 95% CI all included OR=1. The levels being weak in the summer, the analysis according to the quartiles was carried out in winter. No association was observed (results not given).

Statistical analysis (generalized estimating equation models that accounted for autocorrelation of responses, temporal, meteorological, and aerobiological

variables, and some individual characteristics) revealed significant associations between PM_{10}, 0_3, and incident asthma attacks. Odds Ratio (OR) for an increase of 10μg/m^3 of PM_{10} was 1.41; 95% confidence interval (CI) [1.16;1.71]. An increase of 10 μg/m^3 of 0_3 was significantly associated with asthma attacks; OR = 1.20; 95% CI [1.03;1.41]. These relations were observed after a delay between exposure and asthma attacks of 3 to 5 days for PM_{10} and 2 days for 0_3, and they tended to differ according to atopic status. The effect of PM was highly significant in winter but did not appear in summer. The relation with particles was higher for asthma attacks for which no explanatory factor was noted compared to asthma attacks for which a cause was suspected, especially for asthma attacks without infection compared to asthma attacks with infection. Patients taking or not taking oral steroids were equally sensitive to the influence of PM_{10}.
No effect of NO_2 was found on the incidence of asthma attacks over the whole period of study, over both winter and summer periods, whatever the lag after exposure.

Author's Conclusions:

"No effect of SO_2 was found in our study, presumably due to the fact that ambient concentrations have greatly decreased over recent decades, following regulations on air quality. Annual average levels of SO_2 have decreased by more than 85% in Paris and its suburbs in 35 years. In other countries, such as the United Kingdom and countries in Eastern Europe, authors have observed an impact of SO_2 on pulmonary function and on the bronchial hyperreactivity of asthmatic adults. However, SO_2 levels were higher, particularly in Eastern Europe where WHO value guides were exceeded from 35 to 145 times per winter. Our panel study has quantified the short term effects of various urban atmospheric pollutants on asthma attacks for 60 adults suffering from moderate to severe asthma and receiving treatment adapted to the severity of their asthma. All data collected and analyzed show clear effects of PM_{10} and O_3."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitorsWas the exposure concentration measured? (+) study period 1 year Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (+) singleWas the type of blinding indicated? (*i.e.*, single, double, triple, etc) (more info needed) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)

E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (more info needed) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (No) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (No specific conclusions re SO_2) Do the conclusions make biological sense? (No extrapolations re SO_2 made) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no significant results presented for the association between SO_2 and asthma attacks. However, this lack of association could be due to problems in the study design.

This study was based on previous studies but attempted to test the hypothesis that there were consistent and significant associations between pollutants and asthma attacks. The challenge to this study was the inherent bias built into the investigation: each patient was relied on to fill out a daily questionnaire on his or her symptoms. Results from this study suggest that asthma sufferers may wish to adjust medication levels depending on pollutant levels.

No association was observed with SO_2 due to what is assumed to be low ambient concentrations. The effects of NO_2, PM_{10}, and O_3 were discussed as they were quantifiable for the 60 subjects suffering from moderate to severe asthma.

Confidence index:

The subjects were blinded with respect to the objective of the study.

SO_2 measurements were collected at 28 stations. There was no mention of the variability between stations.

There was no information on the distributions of the monitoring stations.

The monitors used in the study were calibrated every two weeks and the method of measurement was detailed in the study.

The subjects were examined at regularly scheduled and emergency doctor visits. While the assessment would be more sensitive than diaries, visits averaged 406 (31/month) which may not have been sufficient to obtain statistical power.

Thirty one percent of the subjects were current or former smokers.

The severity of asthma was variable among the subjects.

The analysis according to the quartiles was carried out in the winter, but the data were not shown.

The study considers only the most severe cases.

This study lacks a proper control and raw data to backup the analyzed data. By considering only the most severe crises, the study could be less sensitive. The results from this study are in line with previous ecological studies, which show the effect of particles on the incidence of asthma attacks.

Review – Confidence index ranking:

Moderate

DRAFT

CASE-CONTROL REVIEW FORM

Author: Wietlisbach, V., Pope, C.A., Ackermann-Liebrich, U.	**Internal ID:** 403

Title: Air Pollution and daily mortality in three Swiss urban areas

Year: 1996

Abstract: Particulate air pollution, in association with other common urban air pollutants, has been associated with various measured health endpoints, including the incidence and duration of respiratory symptoms, lung function, absence from work or school due to respiratory illness, hospitalization for respiratory disease, and cardiopulmonary disease mortality. In this study, the association between daily mortality and air pollution was assessed in Zurich, Basle, and Geneva (Switzerland) for the time period 1984 through 1989. Various regression modeling techniques were used to estimate the effect of air pollution on mortality, to control for time trends, seasonal factors, and weather variables, and to assess the sensitivity of the results. A positive, statistically significant association between daily mortality counts and measures of ambient air pollution in all three cities was observed. Mortality was associated with total suspended particulate pollution, sulfur dioxide, and nitrogen dioxide. The strongest association was with a 3-day moving average (including the concurrent day and the preceding 2 days) of these pollutants. The estimated mortality-air pollution effects were not highly sensitive to regression modeling techniques used to control for seasonality, long-term trends, and weather variables.

Objective: In this study daily mortality and air pollution data for three large Swiss urban areas were analyzed. This study had several specific objectives: 1.to test the hypothesis that daily mortality counts are associated with ambient concentrations of particulate air pollution; 2. to evaluate the sensitivity of the estimated pollution mortality effect to various regression modeling techniques; 3. to evaluate the potential impact of confounding by time trends, seasonal factors, other long wavelength trends, or weather variables; 4. to weigh the evidence that the effect is due to alternative pollutants, and 5. to explore the linearity of the dose-response relationship between pollution and mortality.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean SO_2 levels: Zurich: $35.4 \pm 35.5\ \mu g/m^3$ Basle: $26.5 \pm 25.3\ \mu g/m^3$ Geneva: $40.2 \pm 32.7\ \mu g/m^3$	Study period: 1984-1989	Male and female	All ages	Total mortality for 3 cities: 8.8-21.7/day	No info	n/a	n/a

Observations:

Total mortality was significantly associated with SO_2 in Basle and Geneva. Mortality in $\geq$65y was significantly associated with SO_2 in Basle and Geneva. Respiratory mortality was significantly associated with SO_2 in Zurich and Geneva. Cardiovascular mortality was significantly associated with SO_2 in Basle and

Geneva.

For SO_2, the association with mortality was highly nonlinear with declining marginal effects at higher levels of SO_2. In Basle, at the highest levels of SO_2, the association between SO_2 and mortality is negative.

Statistically significant positive associations between mortality counts and particulate pollution were observed even after controlling for seasonality and daily differences in temperatures and relative humidity. Regression models that included air pollutants were estimated. Mortality was associated with TSP, SO_2, and NO_2. For SO_2, the association mortality was highly nonlinear with declining marginal effects at higher levels of SO_2. Correlation was found between TSP and SO_2. In Zurich and Basle, the Pearson correlation coefficients between TSP and SO_2 equal 0.62 and 0.67 respectively. However, for higher levels of SO_2, the correlation with TSP was weaker.

Author's Conclusions:

"There is little evidence that the mortality-air pollution effects observed were due to the statistical modeling technique used. Simple plots of mortality counts over particulate pollution levels illustrate that there is a small subtle association between mortality and air pollution. The results of this analysis cannot conclusively establish which pollutants are specifically responsible for the observed mortality effects. Mortality was similarly associated with TSP, SO_2, and NO_2. It remains possible that the true culprit pollutant is a constituent of TSP such as inhalable particles, fine or ultra-fine particles, combustion particles, or sulfate particles. This study provides little evidence of a safe threshold level of particulate or related pollutants. The estimated mortality-air pollution effects were not highly sensitive to regression modeling techniques used to control for seasonality, long-term trends, and weather variables."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitors Was the exposure concentration measured? (+) study period 1984 - 1989Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (no info)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)

E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) mortality Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (more info needed) Were accepted assessment methods employed? (more info needed) Were assessment measures sufficiently sensitive? (n/a mortality counts) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) mortality counts Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (more info needed) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (no extrapolations) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Positive statistically significant associations between daily mortality and SO_2 were observed in all of the cities. There was little evidence that the mortality air pollution effects observed were due to the statistical modeling technique used. However, the results of this analysis cannot conclusively establish which pollutant is specifically responsible for the observed mortality effects and may suggest that the pollutants measured could be serving as proxy variables for unknown or

unmeasured pollutants.
The estimated mortality-air pollution effect was not highly sensitive to the type of regression model used in the analysis to control for seasonality, other long-term trends, and daily differences in temperature and relative humidity. However, seasonality was corrected for and the estimated pollution-mortality effect was quite stable to the inclusion or exclusion of other variables such as temperature and relative humidity.

Confidence index:
The sensitivity of different regression models were tested.
Confounders were controlled for in the models.
The objectives were very clear and the method section was very detailed.
Excellent description of study areas.
Good description and explanation of regression models.
No RR or confidence intervals were reported.
Very limited discussion on SO_2 results.
Basic regression modeling approaches were effectively used to evaluate the potential impact of confounding of time trends, seasonal factors and weather variables, among others.

Review – Confidence index ranking:

Moderate to low

CASE-CONTROL REVIEW FORM

Author: Kunii, O., Kanagawa, S., Yajima, I., Hisamatsu, Y., Yamamura, S., Amagai, T., Ismail, I.T.S.	**Internal ID:** 405

Title: The 1997 Haze Disaster in Indonesia: its Air Quality and Health Effects

Year: 2002

Abstract: In this study, the authors assessed air quality and health effects of the 1997 haze disaster in Indonesia. The authors measured carbon monoxide, carbon dioxide, sulfur dioxide, nitrogen dioxide, ozone, particulate matter with diameters less than or equal to 10 microm, inorganic ions, and polycyclic aromatic hydrocarbons. The authors also interviewed 543 people and conducted lung-function tests and determined spirometric values for these individuals. Concentrations of carbon monoxide and particulate matter with diameters less than or equal to 10 microm reached "very unhealthy" and "hazardous" levels, as defined by the Pollution Standards Index. Concentrations of the polycyclic aromatic hydrocarbons were 6-14 times higher than levels in the unaffected area. More than 90% of the respondents had respiratory symptoms, and elderly individuals suffered a serious deterioration of overall health. In multivariate analysis, the authors determined that gender, history of asthma, and frequency of wearing a mask were associated with severity of respiratory problems. The results of our study demonstrate the need for special care of the elderly and for care of those with a history of asthma. In addition, the use of a proper mask may afford protection.

Objective: In the current study, we assessed air quality, investigated overall health effects and factors that influenced the severity of respiratory problems, and determined the affected community's perceptions and practices in response to the haze in Indonesia.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
SO_2 level was reported to be 0.01ppm at each of 3 sites	**Study period: Sept 1997- Nov 1997**	**Male and female**	**No info**	**N=543 (only every 4th subject was examined for health effects N=138)**	**No info**	**n/a**	**n/a**

Observations:

The levels of SO_2were reported, but an analysis of any association between $SO_2$and health effects, perception and preventive behavior was not done. During a few months when a haze was present, SO_2 was at approximately 0.01ppm and subjects reported how they felt during this time during a 10 – 15 minute interview and every 4th subject an examination was performed once.

Physical examination revealed the presence of conjunctivitis in 33.3% of the respondents, wheezing in 8.7% of respondents, and other abnormal respiratory sounds in 2.9% of the respondents. In lung function tests, a restrictive respiratory functional pattern; an obstructive pattern; and both patterns were found in 68.2%, 38.6^, and 22.7%, respectively, of respondents. Whereas there was no significant difference in percentage FVC between age groups and male and female groups, elderly individuals had a significantly lower $FEV_{1.0}$ than younger individuals. Individuals with severe mucus production had a significantly lower $FEV_{1.0}$

than individuals with mild sputum production. Individuals with severe breathlessness when they worked hard had a significantly lower $FEV_{1.0}$ than individuals with mild breathlessness; individuals with severe breathlessness had a significant lower FVC than those with mild breathlessness. Subjects who had a wheezing respiratory sound by auscultation had a significantly lower $FEV_{1.0}$ than those who did not have wheezing sounds.

In multivariate analysis, the authors determined that gender, history of asthma, and frequency of wearing a mask were associated with severity of respiratory problems.

Author's Conclusions:

"There is little evidence about long-term health effects of biomass-generated air pollution. Investigators need to evaluate the development of chronic respiratory diseases and incidence of lung cancer, as well as the longterm change of overall and disease-specific mortality."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) more info needed Are the subjects a representative sample of the population to which extrapolations will be made? (no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitorsWas the exposure concentration measured? (+) 3 month study period Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (no blinding) Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (-) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (-) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (-) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? no info Did qualified personnel conduct the exams?

	(-) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (n/a no statistical analysis re SO_2) Did the investigator appropriately interpret the statistics? (tests for significance no performed) Are the observed trends statistically significant?
G. Interpretations:	(-) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (no extrapolations) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There was no analysis of the association between SO_2 levels and health effects.

Confidence index:

There was no analysis of the association between SO_2 levels and health effects.

The levels of the SO_2 at each of three sites was reported to be .01 ppm, there was no mention of the MDL.

Subjects were not representative of the general population.

Subjects were interviewed during a haze period and basically asked questions during a 10 -15 min interview on past health and how the haze was presently affecting their health. There is likely recall bias, and bias due to the fact that there was a forest fire during the study period. Subjects were not blinded.

The study period was only 3 months.

There is no information on the SO_2monitors.

The study reported frequency of symptoms, perceptions of haze effects and some health effects but did not do an analysis with SO_2

This study described in detail the recruitment process, and the use of convenience sampling to select subjects. The criteria interview was also fully described. The number of subjects used is adequate to extend findings from this study to the larger population.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Desqueyroux, H., Pujet, J.C., Prosper, M., Le Moullec, Y., Momas, I.	**Internal ID:**406
Title: Effects of Air Pollution on Adults with Chronic Obstructive Pulmonary Disease	
Year: 2002	
Abstract: Few studies have been conducted on the effects of air pollution on patients with chronic obstructive pulmonary disease (COPD). During a 14-mo period, 39 Parisian adults with severe COPD were monitored by their physicians. Daily levels of 4 air pollutants were provided by an urban air-quality network. Exacerbation of COPD was associated only with ozone (O_3) (odds ratio [OR] = 1.44 for a 10-microg/m^3 increase in O_3; 95% confidence interval [CI] = 1.14, 1.82), with a lag of 2-3 days. The effect of O_3 was greater in patients whose carbon dioxide pressure ($PaCO_2$) was higher than 43 mm Hg (OR = 1.83; 95% CI = 1.36, 2.47) vs. those with a lower $PaCO_2$ (OR = 1.26; 95% CI = 0.90, 1.77). The effect of O_3 was unchanged, regardless of the maintenance medications used. The only air pollutant to which patients with severe COPD were particularly sensitive was O_3.	
Objective: We studied a panel of adults who suffered from COPD to test their sensitivity to air pollution in Paris.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Summer Mean:7±5μg/m^3 Range: 2-27μg/m^3 Winter Mean:19±12μg/m^3 Range:3-81μg/m^3	Study period: 14 months	10 women 29 men	Average age: 67yr±9yr	39	COPD	n/a	n/a

Observations:

No association was evident between episodes of symptom exacerbation and mean 24hr episodes of symptom exacerbation and mean 24hr concentrations of SO_2 regardless of the lag. Analysis by season did not highlight an OR significantly different from 1. These ORs were calculated for an increase of SO_2 of 10 μg/m^3. Daily variations in SO_2 and O_3 levels followed seasonal patterns. SO_2 concentrations increased during the winter and O_3 concentrations increased in summer. Environmental data was strongly intercorrelated. A highly significant positive correlation was seen among PM_{10}, NO_2, and SO_2, regardless of the season. The temperature was generally associated negatively with the atmospheric pollutants SO_2, PM_{10}, and NO_2, and positively with O_3.

No association was evident between episodes of symptom exacerbation and mean 24-hr concentrations of SO_2, PM_{10}, and NO_2, and the maximum 1hr level of NO_2, regardless of lag.

Author's Conclusions:

The only air pollutant to which patients with severe COPD were particular sensitive was O_3.

In summary, although O_3 was related to COPD exacerbation in our panel, no association was observed for the other pollutants studied. The severely affected patients in our panel were exposed to low levels of ambient outdoor pollutants that resulted from their reduced mobility and ventilatory flow. Perhaps their therapeutic treatment is sufficient for the prevention of main disorders that could be caused by slight fluctuations in ambient air pollution concentrations - with the possible exception of O_3, which could be more aggressive.

"In our study, no association was found between COPD and PM, SO_2, or NO_2. The only significant results with ambient SO_2 concentrations were produced in ecological studies in Europe. It is important to note that some of the associations highlighted, particularly in ecological studies, results from 1-pollutant models and did not test the simultaneous influence of several pollutants. There is a risk of attributing an effect to 1 pollutant that should, in fact be ascribed to another because of the colinearity that exists among several urban air pollutants."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitorsWas the exposure concentration measured? (+) study period 14 months Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale?

	(+) Did qualified personnel conduct the exams? **(more info needed)** Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+/-)Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(no conclusions re SO_2)** Do the conclusions make biological sense? **(no extrapolations reSO_2)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study examined confounding and modifying factors individually, in order to collect information on factors that were likely responsible for episodes of symptom exacerbation. Findings suggest that there is no association between COPD and SO_2, inconsistent with ecological studies in Europe. The author points out that ecological studies might not test simultaneous influences of several pollutants, and attribute ensuing health effects tospecific pollutants.

A statistically significant association was found between O_3 and the exacerbation of symptoms in COPD patients.

Confidence index:

The cases were selected by 2 pulmonary physicians. Only crises verified by a physician were considered and therefore the study could have suffered from a lack of power.

Patients were asked to consult their pulmonary physician at the clinic when they experience exacerbations of their symptoms. Therefore, the results depended on the reliability of the patients to visit every time they experienced health effects.

Most of the subjects were on regular medication which could make them less sensitive to pollution.

The tables of results were very detailed and descriptive.

Some advantages of the study included homogeneity of patients with regard to age and severity of COPD, evaluation of the medical variables and confounders on an individual scale and effects verified by specialists.
Limited detail on monitoring systems.
This study incorporated confidence intervals and effectively used models to produce statistically significant findings. The subject recruitment process was clearly delineated, and the authors used questionnaires for inclusion purposes, gathering information on age, gender, residence and occupation. However, the findings from the surveys were not fully included in the study.
This study was based on observations made on 39 COPD patients. No control experiments were done and individual and raw data was not reported in the paper.

Review – Confidence index ranking:

Moderate to low

CASE-CONTROL REVIEW FORM

Author: Le Tertre, A., Quenel, P., Eilstein, D., Medina, S., Prouvost, H., Pascal, L., Boumghar, A., Saviuc, P., Zeghnoun, A., Filleul, L., Declercq, C., Cassadou, S., Le Goaster, C.	**Internal ID:**407

Title: Short Term Effects of Air Pollution on Mortality in Nine French Cities: A Quantitative Summary

Year: 2002

Abstract: Between 1990 and 1995, 9 French cities provided data on daily air pollution, total mortality, cardiovascular mortality, and respiratory mortality. Personnel in individual cities performed Poisson regressions, controlling for trends in seasons, calendar effects, influenza epidemics, temperature, and humidity, to assess the short-term effects of air pollution. The authors describe results obtained from the quantitative pooling of these local analyses. When no heterogeneity could be detected, a fixed-effect model was used; otherwise, a random-effect model was used. Significant and positive associations were found between total daily deaths in these cities and the 4 air pollution indicators studied: (1) Black Smoke, (2) sulfur dioxide, (3) nitrogen dioxide, and (4) ozone. A 50-microg/m^3 increase in Black Smoke (24 hr), sulfur dioxide (24 hr), nitrogen dioxide (24 hr), or ozone (8 hr) was associated with increases in total mortality of 2.9% (95% confidence interval [CI]) = 1.3, 4.4), 3.6% (95% CI = 2.1, 5.2), 3.8% (95% CI = 2.0, 5.5), and 2.7% (95% CI = 1.3, 4.1), respectively. Similar results were obtained for cardiovascular mortality. Except for sulfur dioxide, positive--but not significant--associations were found with respiratory mortality. The internal consistency among the cities studied, as well as consistency with previously published results, favors a causal interpretation of these associations.

Objective: The investigators of the French 9-cities project, conducted between 1990 to 1995 attempted to quantify the short-term effects of air pollution on mortality in medium-sized cities.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Actual levels not provided. However, RR calculated for a 50 µg/m^3 increase in SO_2.	Study period: 1990-1995	Male and female	No info	Mortality counts Total population across n=9 cities: 260,000-6.2 million	No info	n/a	n/a

Observations:

For a 50 µg/m^3 increase in SO_2, all RR for total mortality were greater than 1, although they reached a nominal significance level of 5% in the 4 cities of Bordeaux, Lile, Lyon and Paris. The SO_2 results were heterogeneous across cities for total mortality. There was also heterogeneity in results for SO_2 relative to cardiovascular deaths. Results for cardiovascular mortality were less heterogeneous, but the confidence intervals were wide.

For the pooled results, a 50 µg/m^3 increase of 3.6% in total mortality , 5.3% in cardiovascular mortality and 5.6% in respiratory mortality was observed.

Author's Conclusions:

"In conclusion, the coherence of results across various cities and studies, especially for Black Smoke and O_3-along with what is known of possible mechanisms reinforces our argument that the associations found in this study were most likely causal."

"Our combined analysis summarizes the results from 9 French cities on the short-term effects on mortality of 4 indicators of air pollution. We found that all the pollutants studied had a clear and significant effect on total mortality, with a 50 µg/m^3 increase in pollutants being associated with an increase of 2.7%-3.8% in total daily deaths. Results for cardiovascular mortality showed a significant RR for black smoke, SO_2, and NO_2 ranging from 3.1% to 5.6%."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitorsWas the exposure concentration measured? (+) study period: 1990-1995 Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls0 Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (more info needed) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed?

	(+) **mortality counts** Were assessment measures sufficiently sensitive? **(n/a no symptom analysis)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (-) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+/-) **original objective unclear.** Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(n/a)** Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Significant associations between SO_2 and total mortality, cardiovascular mortality and respiratory mortality were observed. However, the results of the cities were heterogeneous. The RRs were quite low and some of the results that were reported as significant had 95% CIs which contained RR=1.00. The authors conclude that the findings in the study imply causality, but causality cannot be determined from a meta-analysis of panel studies.

Since a few cities could provide data for all pollutants and some provided data for only a brief period, the study was unable to explore possible effect modifiers.

This study found that all the pollutants studied, including SO_2, have clear short-term effects on mortality. There is also a definite link between cardiovascular mortality and SO_2. Results for respiratory mortality were less significant.

Confidence index:

There was heterogeneity between the cities.

SO_2 levels were not available for all cities for the whole period of study. The populations varied significantly between cities. Paris had the largest population and results were calculated with and without the Paris data. It was determined that the Paris data was significantly controlling the results. One of the cities which showed significantly higher RR, also used a different method to determine SO_2. Authors report a clear and significant effect of all pollutants. However, many of the 95% CI contained RR=1. More detail is needed on the equipment and monitoring. Mortality data was collected from the national statistics. Data was differentiated according to cause of death, but information such as previous health and socioeconomic data was not provided. Collecting monitoring data from multiple cities was complicated by the fact that some stations provided data on only one pollutant, while other provided data on multiple pollutants. To correct for this, the authors conducted sensitivity analysis to determine if changes occurred when data was excluded.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Ha, E.H., Lee, J.T., Kim, H., Hong, Y.C., Lee, B.E., Park, H.S., Christiani, D.C.	**Internal ID:**408

Title: Infant Susceptibility of Mortality to Air Pollution In Seoul, South Korea

Year: 2003

Abstract: OBJECTIVE: Susceptibility of target populations to air pollution is an important issue, because air pollution policies and standards should be based on the susceptibilities of those at particular risk. To evaluate which age group is more susceptible to the adverse health effects of air pollution, we compared the effects of air pollution on mortality among postneonates, those aged 2 to 64 years, and those over 65 years of age. DESIGN: Daily counts of total and respiratory death along with daily levels of meteorological variables and air pollutants were analyzed using generalized additive Poisson regression. The relative risks (RR) of mortality for interquartile changes of the levels of particulate matter <10 µm (PM_{10}) were calculated on the same day. RESULTS: For postneonates, the RR of total mortality for an interquartile change (42.9 $\mu g/m^3$) in PM_{10} (RR: 1.142; 95% confidence interval [CI]: 1.096-1.190) was greatest among age groups. Next were the elderly over 65 years of age (RR: 1.023; 95% CI: 1.022-1.024). Regarding respiratory mortality, RR for an interquartile change of PM_{10} in postneonates (RR: 2.018; 95% CI: 1.784-2.283) was also greater than those in the other groups. CONCLUSIONS: These results agree with the hypothesis that infants are most susceptible to PM_{10} in terms of mortality, particularly respiratory mortality.

Objective: Susceptibility of target populations to air pollution is an important issue, because air pollution policies and standards should be based on the susceptibilities of those at particular risk. To evaluate which age group is more susceptible to the adverse health effects of air pollution, we compared the effects of air pollution on mortality among postneonates, those aged 2 to 64 years, and those over 65 years of age..

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean: 11.1±7.0 ppb Range: 2.4-46.0ppb Interquartile range: 7.8ppb	Study period: 1995-1999	Male and female	All ages	Total deaths: postneonates-1045 2-64 yrs-67597 >65yrs-100,316	No info	n/a	n/a

Observations:

For total mortality, the RRs for an 7.8 ppb increase in SO_2 were greater than 1 in Postneonates and >65y.
For respiratory mortality, the RRs for a 7.8 ppb increase in SO_2 were greater than 1 for persons 2-64 years old.
The concentrations of CO, NO_2, SO_2, and PM_{10} were positively correlated with each other. However, the concentration of O_3 was negatively correlated with the other pollutants.
For postneonates, the RR of total mortality for an interquartile change in PM_{10} was greatest among age groups. Next were the elderly over 65 years of age.
Regarding respiratory mortality, RR for an interquartile change of PM_{10} in postneonates was also greater than those in the other groups.

Author's Conclusions:

There were no specific conclusion for SO_2associations and mortality.

"We hypothesized that infants are more vulnerable to respiratory disease leading to death from particulate air pollution, because the infant lung and immune system is immature and unable to control adequately the inflammation resulting from exposure to ambient particle.

Low temperature could be associated with excess deaths as well as air pollution episodes, confounding the association between air pollution and mortality. We controlled the short-term and prolonged effects of low temperature by adding the temperatures of the same day in addition to the seasonal factor in the analysis.

To our knowledge, this is the first study to determine that infants are the most susceptible age group after directly comparing with other age groups. This result has serious implications on the air pollution criteria, which should be based on the effects on infant health rather than adult health."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed moniotorsWas the exposure concentration measured? (+) study period Jan 25, 1995 – December 1999. Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) mortality Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) mortality Were assessment measures sufficiently sensitive?

	(n/a no symptom analysis) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? **(No interpretation)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(-) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? **(more info needed)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(no specific conclusions re SO_2)** Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(No)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There are positive associations observed between $SO_2$and mortality for some of the age groups. However, the results are not discussed in the study.

Some of the limitations associated with this study include the fact that environmental monitoring data do not necessarily represent individual exposures. Furthermore, individual risk factors, such as underlying disease or exposure to smoking were not considered in the analysis.

This study showed that the increase in respiratory mortality was associated with pollutant exposure, and the risk estimates were largest in postneonates particularly with respiratory mortality. Also, there is increased risk with those over the age of 65. The increase in risk for children is possibly due to the fact that infant lung and immune system is immature and unable to control adequately the inflammation resulting from exposure.

Confidence index:

The focus of the study is PM_{10}. All of the figures etc. look at PM_{10}, even though the description of the figures states the figure is for all pollutants. Limited

analysis is given for SO_2. The results for SO_2 are not discussed at all.
The age grouping may be an issue. The second group consisted of people 2-64 yrs old and should have been stratified further.
Cause of mortality may be misdiagnosed in very young children.
There is limited information on the statistical analysis.
Post neonates will likely spend most of their time indoors.
Confounders such as influenza were not considered.
This study appreciated that different age groups may respond differently to air pollution, dividing the sample group into three groups based on age. The daily records of mortality used to provide data in this study also reported date of birth and place of residence. This study utilized 27 monitoring sites for measurement of air pollutants.
This study places emphasis on particulate matter and carbon monoxide. Some weaknesses in this study are possible misrepresentation in individual exposures and underlying diseases were not considered in this study. In the discussion, the authors also point out that the main cause of postneonatal death was congenital anomalies, which could confound the association between air pollution and mortality.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Jaffe, D.H., Singer, M.E., Rimm, A.A.	**Internal ID:**409

Title: Air Pollution and emergency department visits for asthma among Ohio Medicaid recipients, 1991-1996

Year: 2003

Abstract: We examined the effects of nitrogen dioxide (NO_2), ozone (O_3), particulate matter of <10 μm aerodynamic diameter (PM_{10}), and sulfur dioxide (SO_2) on asthmatics ages 5-34 years enrolled in Medicaid in Cincinnati, Cleveland, and Columbus, OH (N=5416). Our study period was for the summer months, June-August, from July 1, 1991 to June 30, 1996. We preformed Poisson regression analyses for the number of daily emergency department (ED) visits for asthma in each city and on the aggregate data controlling for time trends and minimum temperature. We found a 12% increased likelihood of an asthma ED visit per 50 $\mu g/m^3$ increase in PM_{10} in Cleveland [95% confidence interval (CI)=0-27%] and a 35% increase per 50 $\mu g/m^3$ increase in SO_2 in Cincinnati (95% CI=9-21%). When data were analyzed for all three cities combined, the risk of an ED visit increased for all pollutant increases and specifically by 12% (95% CI=1-23%) per 50 $\mu g/m^3$ increase in SO_2. Attributable risk estimates show a five times greater impact on Cleveland over Cincinnati or Columbus. Between 1991 and 1996, air pollutants in Cincinnati, Cleveland, and Columbus increased ED visits for asthmatics enrolled in Medicaid.

Objective: The main objective of this study is to assess the short-term relationship between outdoor air pollution and asthma ED visits among Medicaid participants, where community-level insurance status and economic conditions are relatively uniform. Our secondary objective was to quantify the impact of exposure on each community by calculating attributable risks.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Average daily SO_2: 35.9±25.1 ug/m^3 (24h mean in Cincinnati) 39.4±25.3 ug/m^3 (24h mean in Cleveland) 11.1±8.5 ug/m^3 (24h mean in Columbus)	Study period: 1991-1996	Male and female	5-34 y	4416	Asthmatic	n/a	n/a

Observations:

Risk estimates from SO_2 exposure were positive for all three cities. Asthma visits increased from 6% in Cleveland to 35% in Cincinnati per 50 $\mu g/m^3$ increase in SO_2. Results from aggregate data of all three cities resulted in an overall increased risk of an emergency dept. episode per 50 $\mu g/m^3$ increase of SO_2 of 12% (95% CI 1-23%).

No consistent dose response relationship was observed for SO_2.
The attributable risk for an asthma ED episode from short-term exposure to increased SO_2 were 4.20, 0.66 and 2.94 for Cincinnati, Cleveland and Columbus respectively (per 10,000 people)
Regression models assessing exposure thresholds for each pollutant in each city were performed. No consistent effects for all three cities were observed for SO_2.

Author's Conclusions:

These findings reinforce the importance of air pollution controls and asthma prevention especially in poor, urban populations.
"Between 1991 and 1996, air pollutants in Cincinnati, Cleveland, and Columbus increase ED visits for asthmatics enrolled in Medicaid."
"Despite the limitations of an ecological study and the use of secondary data, our findings mostly corroborate the results of other investigators of the effects of low levels of ambient air pollutants on asthmatic episodes in the ED. The air pollution - asthma association remains positive even after controlling for insurance and economic status. These findings reinforce the importance of air pollution controls and asthma prevention especially in poor, urban populations."
"Exposures to O_3 and SO_2 increased the number of ED asthma episodes for Cincinnati, Cleveland, and Columbus when exposure lagged by 2 or 3 days. These results agree with many of the findings in the literature, although comparability is limited to those studies that employed exposure periods. Regression estimates for SO_2 produced relatively wide confidence intervals that may have been caused by interaction with copollutants not evaluated in the regression models or other uncontrolled confounding variables such as pollen or flu epidemics."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitorsWas the exposure concentration measured? (+) study period 1990 – 1996 Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)

E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? **(more info needed)** Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (no info) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(n/a no specific conclusions re SO_2)** Do the conclusions make biological sense? **(no extrapolations)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Positive associations between SO_2 and asthma episodes were observed among Medicaid-enrolled asthmatics in three urban cities in Ohio. The study found a 35% increase per 50 μg/m^3 increase in SO_2 in Cincinnati **(95% CI=9-21%)**. However, this result is not included in the confidence interval. When data were analyzed for all three cities combined, the risk of an ED visit increased specifically by 12% (95% CI=1-23%) per 50 μg/m^3 increase in SO_2. Attributable risk estimates showed a five times greater impact on Cleveland over Cincinnati or Columbus.

This study looked at the short-term relationship between outdoor air pollution and asthma visits among Medicaid participants where community-level insurance status was relatively uniform. Findings suggested a positive association between outdoor air pollutants and asthma episodes among Medicaid-enrolled asthmatics.

The findings of this study mostly corroborate the results of other investigators of the effects of low levels of ambient air pollutants on asthmatic episodes in the emergency department. The air pollution-asthma association remains positive even after controlling for insurance and economic status. These findings reinforce the importance of air pollution controls and asthma prevention especially in poor, urban populations.

Confidence index:

There appears to be an error in the results reported for Cincinnati The study found a 35% increase per 50 μg/m^3 increase in SO_2 in Cincinnati **(95% CI=9-21%)**. However, this result is not included in the confidence interval.

A relatively low rate of ED asthma episodes was observed per day during the study period. These visits for asthma might only represent a fraction of those experiencing asthma exacerbations but who require emergency noninpatient treatment.

Regression estimates for SO_2 produced relatively wide confidence intervals that may have been caused by uncontrolled confounding variables such as pollen or flu epidemics.

The results were variable between the three cities.

The recruitment process in this study was effectively described in this study. Rationale for exclusion was described. The age criterion (5-34) restricted the study to individuals who were not babies, nor the elderly, two groups that might be immunocompromised and might bias the findings.

In all of these case-control studies, one must also be aware of misclassification of both exposure and symptoms.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Hajat, S., Anderson, H.R., Atkinson, R.W., Haines, A. | **Internal ID:**410

Title: Effects of air pollution on general practitioner consultations for upper respiratory diseases in London

Year: 2002

Abstract: OBJECTIVES: Few published studies have examined the effect of air pollution on upper respiratory conditions. Furthermore, most epidemiological studies on air pollution focus on mortality or hospital admissions as the main health outcomes, but very rarely consider the effect in primary care. If pollution effects do exist then the public health impact could be considerable because of the many patient contacts involved. We investigated the relation between air pollution and upper respiratory disease as reflected in number of consultations made at family practices in London. METHODS: The study used non-parametric methods of analysis of time series data, adjusting for seasonal factors, day of the week, holiday effects, influenza, weather, pollen concentrations, and serial correlation. RESULTS: It was estimated that a 10-90th percentile change (13-31 microg/m^3) in sulphur dioxide (SO_2) measures resulted in a small increase in numbers of childhood consultation: 3.5% (95% confidence interval (95% CI 1.4% to 5.8%). Stronger associations were found in the case of a 10-90th percentile change (16-47 microg/m^3) in fine particles (PM_{10}) in adults aged 15-64 5.7% (2.9% to 8.6%), and in adults aged 65 and over: 10.2% (5.3% to 15.3%). In general, associations were strongest in elderly people, weakest in the children, and were largely found in the winter months for these two age groups, and in the summer months for adults aged 15-64. An apparent decrease in consultations was associated with ozone concentrations but this was most pronounced in colder months when ozone concentrations were at their lowest. CONCLUSIONS: The results suggest an adverse effect of air pollution on consultations for upper respiratory symptoms, in particular in the case of PM_{10} and SO_2. The effects are relatively small; however, due to the many consultations made in primary care, the impact on demand for services could be considerable.

Objective: Few published studies have examined the effect of air pollution on upper respiratory conditions. Furthermore, most epidemiological studies on air pollution focus on mortality or hospital admissions as the main health outcomes, but very rarely consider the effect in primary care. If pollution effects do exist then the public health impact could be considerable because of the many patient contacts involved. We investigated the relation between air pollution and upper respiratory disease as reflected in number of consultations made at family practices in London.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean SO_2: Warm Season 20.5±6.5µg/m^3 Cool Season 22.0±9.0 µg/m^3	Study period: 1992-1994	Male and female	0-14y; 15-64y; and ≥65y	Average number of daily consultations: 73.9 ± 50.2 for young; 96.3±62.6 for adults and 15.5±11.3 for elderly	No info	n/a	n/a

Observations:

The percentage change in the number of consultations for upper respiratory disease was calculated by age group and season for a specific increase in SO_2 (15µg/m^3 for the warm season; 20.5µg/m^3 for the cool season and 18µg/m^3 for all year). The following observations were statistically significant $p<0.01$. For the young, the percentage change in number of consultations were significant for all year (3.5% (1.4-5.8) lag 0) and for the cool season (5.5% (2.4-8.7) lag 0). For the adults, the percentage change was significant for the warm season (4.6% (1.5-7.7). Finally, for the elderly, the percentage changes were not significant. Stronger associations were found in the case of a 10-90th percentile change (16-47 ug/m3) in fine particles (PM10) in adults aged15-64 5.7% (2.9% to 8.6%), and in adults aged 65 and over: 10.2% (5.3% to 15.3%).

The exposure response plots of the relative risk of consulting for upper resp. disease in adults was presented against the concentrations of SO_2. It suggested that there were many days where SO_2concentrations were associated with an increased relative risk of consultations for upper respiratory disease. For SO_2, there appeared to be a threshold of approximately 20 µg/m^3 (from the graph).

In general, associations were strongest in elderly people, weakest in children, and were largely found in the winter months for these two age groups, and in the summer months for adults aged 15-64. An apparent decrease in consultations was associated with ozone concentrations but this was most pronounced in colder months when ozone concentrations were at their lowest.

Author's Conclusions:

"In summary, our results suggest an acute effect of low concentrations of air pollution on upper respiratory diagnoses, in particular with PM_{10} and SO_2, and shows that primary care is a feasible source of data for studies of this type."

"The effects are relatively small; however, due to the many consultations made in primary care, the impact on demand for services could be considerable."

"Our results suggest a consistent association between low concentrations of air pollution and consultations for upper respiratory disease, in particular with SO2 in children and PM_{10} and NO_2 in elderly people and the adults. This agrees with the study by Jaakkola et al. showing that, for children, air pollution can be hazardous at relatively low concentrations. Clinical evidence suggests that pollutants are deposited during inspiration through the nose, and are absorbed in the nasal mucosa, resulting in several deleterious effects on the body."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitors Was the exposure concentration measured? (+) Study period 1992-1994 Is the exposure duration known?

C. Equipment:	(-) Was a full description of the monitoring devices provided? (No info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) **but more info needed** Do the conclusions make biological sense? **(no extrapolations)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Acute effects of SO_2 were found on consultations for upper respiratory disease. PM_{10} and SO_2 were consistently associated with an increase in consultations. These effects were found at relatively low concentrations of SO_2. The effects were small, but due to the many patient contacts in primary care, the impact on demand for services could be considerable. The effects were greatest for children and lowest for the elderly. However, there were significantly lower numbers of daily visits by the elderly.

In determining the relationships that exist between air pollutants and health effects, the authors considered pollen types, which could confuse findings.

Confidence index:

There were five monitoring sites for SO_2. Location and homogeneity of the sites is not given.

Many confounders were considered in the analysis.

The discussion on $SO_2$is very limited.

The results presented in the paper only highlight the most significant associated lag for each pollutant.

Time series of GP consultations are strongly influenced by human behavior and service availability Also, the source of the data in this study does not distinguish between patients making emergency consultations and those coming in for routine prescriptions.

The URD diagnostic category used in the analysis consisted of several different diseases some of which were acute and some of which were chronic.

Some of the confidence intervals were wide.

A very large sample size was utilized in this study. The study attempted to lessen number of subjects used for statistical analysis by dividing the sample into three groups: children (aged 0-14), adults (15-64) and elderly people (65+).

There is the potential for misclassification of both exposure and outcome.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Hong, Y.C., Leem, J.H., Ha, E.H., Christiani, D.	**Internal ID:** 412
Title: PM_{10} Exposure, Gaseous Pollutants, and Daily Mortality in Inchon, South Korea	
Year: 1999	

Abstract: To evaluate the relative importance of various measures of particulate and gaseous air pollution as predictors of daily mortality in Inchon, South Korea, the association between total daily mortality and air pollution was investigated for a 20-month period (January 1995 through August 1996). Poisson regression was used to regress daily death counts on each air pollutant, controlling for time trends, season, and meteorologic influences such as temperature and relative humidity. Regression coefficients of a 5-day moving average of particulate matter less than or = to 10 microm in aerodynamic diameter (PM_{10}) on total mortality were positively significant when considered separately and simultaneously with other pollutants in the model. PM_{10} remained significant when the models were confined to cardiovascular or respiratory mortality. Sulfur dioxide (SO_2) and carbon monoxide (CO) were significantly related to respiratory mortality in the single-pollutant model. Ozone exposure was not statistically significant with regard to mortality in the above models, and graphic analysis showed that the relationship was nonlinear. A combined index of PM_{10}, nitrogen dioxide, SO_2, and CO seemed to better explain the exposure-response relationship with total mortality than an individual air pollutant. Pollutants should be considered together in the risk assessment of air pollution, as opposed to measuring the risk of individual pollutants.

Objective: The objective of our study was to report on the association between daily mortality and ambient air pollution in Inchon, South Korea.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean: 18.0±9.7 ppb Range: 2.4-52.8 ppb	Study period: 20 months	Male and female	No info	Total Population: 2.4 million Total deaths/day: 22.1±4.9	No info	n/a	n/a

Observations:

Pearson correlations between SO_2 and mortality were small but positive and statistically significant both when a 5-day moving average and the previous day's exposure were used. The correlation for current day exposure was not significant.

In the regression analysis, the associations between SO_2 and total mortality were insignificant.

$SO_2$was a significant predictor of respiratory mortality using the previous day's exposure in the single-pollutant model using the previous day's concentrations. At concentrations of 0-40 ppb the log of the relative risk remained near or below 0. A combined index of PM_{10}, nitrogen dioxide, SO_2 and CO seemed to better explain the exposure-response relationship with total mortality than an individual air pollutant.

The index of PM_{10} + SO_2 also showed a near-linear increase of RR, which suggests that the joint effect model of PM_{10} and SO_2 is a better predictor of mortality risk than effect models of individual pollutants. Contour analysis illustrates this joint effect well, showing that when concentrations of the two pollutants were high, the mortality rate peaked.

PM_{10} was correlated significantly with other air pollutants, positively with NO_2, SO_2, and CO, and negatively with O_3.

Author's Conclusions:

"Pollutants should be considered together in the risk assessment of air pollution, as opposed to measuring the risk of individual pollutants."

"In summary, the association of particulate air pollution with daily mortality in Inchon is similar in magnitude to the results in other communities in the United States and in Europe. These associations are still observed when weather variables and other pollutants are accounted for. The association between particulate air pollution and mortality was largest for respiratory deaths. Increased cardiovascular mortality associated with particulate air pollution was also observed. Further, there is no evidence to support a threshold for particulate air pollution. Future studies should be directed at evaluating the mutual interaction of pollutants and their chemical composition."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitorsWas the exposure concentration measured? (+) study period 609 days Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (No info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) mortality Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) mortality Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and

	dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) mortality Were assessment measures sufficiently sensitive? (n/a mortality) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) mortality counts Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (few) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (No specific SO_2 conclusions made) Do the conclusions make biological sense? (No extrapolations re SO_2 made) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Sulfur dioxide was significantly related to respiratory mortality for previous day exposure. Sulfur dioxide levels were not significantly related to total mortality or cardiovascular mortality.

A combined index of PM_{10}, nitrogen dioxide, sulfur dioxide, and carbon monoxide seemed to better explain the exposure-response relationship with total mortality of air pollution, as opposed to measuring the risk of individual pollutants. SO_2 and CO were significantly related to respiratory mortality in the single-pollutant model. Further studies should be directed at evaluating the mutual interaction of pollutants and their chemical composition.

Confidence index:

The main fault of this study is limited detail re. exposure. The location of the two monitors was not given with respect to the population and a full description of the monitoring stations was not given. The deaths were collected from a national register and info re. the location of the subjects' residence was not given. There was no discussion of the SO_2 results in the discussion section or in the conclusions. Tthis study is limited by its use of environmental monitoring data alone to represent ambient concentrations which do not necessarily represent individual exposures

This study relies on annual detail mortality tapes for accurate representation of the number and cause of deaths from January 1995 to August 1996. While the deaths were categorized according to causation, other possible confounding factors such as demographic data, was unaccounted for. This study also did not mention a specific number of subjects that were involved in the study, calling into question the validity of the study findings.

Confidence intervals were not reported in this study.

Review – Confidence index ranking:

Low to moderate

DRAFT

CASE-CONTROL REVIEW FORM

Author: Delfino, R.J., Gong, H., Linn, W.S., Pellizzar, E.D., Hu, Y.	**Internal ID:** 413
Title: Asthma Symptoms in Hispanic Children and Daily Ambient Exposures to Toxic and Criteria Air Pollutants	
Year: 2003	
Abstract: Although acute adverse effects on asthma have been frequently found for the U.S. Environmental Protection Agency's principal criteria air pollutants, there is little epidemiologic information on specific hydrocarbons from toxic emission sources. We conducted a panel study of 22 Hispanic children with asthma who were 10-16 years old and living in a Los Angeles community with high traffic density. Subjects filled out symptom diaries daily for up to 3 months (November 1999 through January 2000). Pollutants included ambient hourly values of ozone, nitrogen dioxide, sulfur dioxide, and carbon monoxide and 24-hr values of volatile organic compounds (VOCs), particulate matter with aerodynamic diameter < 10 microm (PM_{10}, and elemental carbon (EC) and organic carbon (OC) PM_{10} fractions. Asthma symptom severity was regressed on pollutants using generalized estimating equations, and peak expiratory flow (PEF) was regressed on pollutants using mixed models. We found positive associations of symptoms with criteria air pollutants (O_3, NO_2, SO_2, PM_{10}), EC-OC, and VOCs (benzene, ethylbenzene, formaldehyde, acetaldehyde, acetone, 1,3-butadiene, tetrachloroethylene, toluene, m,p-xylene, and o-xylene). Selected adjusted odds ratios for bothersome or more severe asthma symptoms from interquartile range increases in pollutants were, for 1.4 ppb 8-hr NO_2, 1.27 [95% confidence interval (CI), 1.05-1.54]; 1.00 ppb benzene, 1.23 (95% CI, 1.02-1.48); 3.16 ppb formaldehyde, 1.37 (95% CI, 1.04-1.80); 37 $\mu g/m^3$ PM_{10}, 1.45 (95% CI, 1.11-1.90); 2.91 $\mu g/m^3$ EC, 1.85 (95% CI, 1.11-3.08); and 4.64 $\mu g/m^3$ OC, 1.88 (95% CI, 1.12-3.17). Two-pollutant models of EC or OC with PM_{10} showed little change in odds ratios for EC (to 1.83) or OC (to 1.89), but PM10 decreased from 1.45 to 1.0. There were no significant associations with PEF. Findings support the view that air toxins in the pollutant mix from traffic and industrial sources may have adverse effects on asthma in children.	
Objective: In the present study, we address the need to evaluate respiratory health effects of air toxic in communities in proximity to major emission sources.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
SO_2 1-hr max 7.0±4.0 ppb Range: 2-26ppb SO_2 8-hr max 4.6±3.0ppb Range: 1-20 ppb	Study period: 3 months 4Nov99 to 23Jan00	18 males 4 females	Median age: 12 Range: 10-16	22	asthmatic	n/a	n/a

Observations:

Positive associations were observed between symptoms and SO_2 .

SO_2 levels were not associated with PEF values.

The relationship of symptoms in children with asthma to interquartile range increases in SO_2 (4.0 ppb for 1 hr max) and (2.5ppb for 8hr max) were calculated as odds ratios:

	ORs (95% CI) for symptom scores >1	ORs (95% CI) for symptom scores >2
SO_2 1-hr max, lag 0	1.31 (1.10-1.55) ($p<0.01$)	1.37 (0.87-2.18)
Lag 1	1.11 (0.91-1.38)	0.76 (0.35-1.64)
SO_2 8hr max, lag 0	1.23 (1.06-1.41) $p<0.01$	1.36 (1.08-1.71) $p<0.01$
Lag 1	1.11 (0.97-1.28)	0.91 (0.51-1.60)

We found positive associations of symptoms with criteria air pollutants (0_3, NO_2, SO_2, PM_{10}), EC-OC, and VOCs (benzene, ethylbenzene, formaldehyde, acetaldehyde, acetone, 1,3-butadiene, tetrachloroethylene, toluene, m, p-xylene, and o-xylene).

Selected adjusted odds ratios for bothersome or more severe asthma symptoms from interquartile range increase in pollutants were: for 1.4ppb 8-hr NO_2, 1.27 [95% confidence interval (CI), 1.05-1.54]; 1.00 ppb benzene, 1.23 (95% CI, 1.02-1.48); 3.16 ppb formaldehyde, 1.37 (95% CI, 1.04-1.80); 37 $\mu g/m^3$ PM_{10}, 1.45 (95% CI, 1.11-1.90); 2.91 $\mu g/m^3$ EC, 1.85 (95% CI, 1.11-3.08); and 4.64 $\mu g/m^3$ OC, 1.88 (95% CI, 1.12-3.17). Two pollutant models of EC or OC with PM_{10} showed little change in odds ratios for EC (to 1.832) or OC (to 1.89), but PM_{10} decreased from 1.45 to 1.0. There were no significant associations with PEF. NO2 and SO2 were strongly correlated. Positive correlations likely represent common sources from fossil fuel combustion and common meteorologic determinants such as air stagnation. Generally, most pollutants were positively correlated with temperature and negatively correlated with wind speed. Respiratory infection reports were significantly associated with bothersome or more severe asthma symptoms and with asthma symptoms interfering with daily activities. When regressing SO_2 or NO_2 with a VOC compound, moderate to high levels of correlation between pollutant variables led to multicollinearity in regression models, as indicated by variance inflation and reductions in regressing parameters for both coregressed pollutants in most models.

*Statistically significant

Author's Conclusions:

"Findings support the view that air toxics in the pollutant mix from traffic and industrial sources may have adverse effects on asthma in children."
"Some of the VOCs, NO_2, SO_2, OC, and EC may all be markers for a causal mixture of traffic-related pollutants in an area with high traffic density. Our view that criteria pollutant gases function at least partly as surrogate indicators for a causal mixture of toxic air pollutants is supported by our findings that associations for SO_2 and NO_2 were found at very low concentrations relative to concentrations that induce adverse respiratory effects in human clinical studies."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (+) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (-) see explanation belowWas the method of recruitment appropriate? Would the participation incentive employed cause the selection of an

	unrepresentative sample population?
B. Exposure conditions:	(+) **fixed monitors** Was the exposure concentration measured? (+) **study period 3 months** Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(not given)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (-) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) **final exam**Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.,* additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? **(no extrapolations made)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Significant adjusted odds ratios for bothersome or more severe asthma symptoms from interquartile range increases in pollutants were, for 4.0 ppb 1-hr SO_2, 1.31 [95% confidence interval (CI), 1.10-1.55] and for 2.5ppb 8hr SO_2, 1.23 [1.06-1.41] and 1.36[1.08-1.71]. However, these ORs were very low and there were problems in the study design. See below.

This study looked at health effects of air toxics in communities in proximity to major emissions sources. Furthermore, while many studies have examined the associations between asthma and air pollutants, this study attempted to identify which of the air pollutants was responsible for exacerbating asthma.

Results suggest that further study on potentially causal air toxics in the pollutant mix from traffic and industrial sources is needed. Air toxics in the pollutant mix may have adverse effects on asthma in children. This study sheds some light on identifying the causal components and susceptible subgroups.

Confidence index:

Initially the authors set criteria for inclusion into the study. However, when the desired number of subjects were not recruited, additional children were chosen (nonrandom) to bring the number of children to the desired number.

The sample size was too small. Only 16 of the 22 subjects had recorded suitable asthma symptoms for the study. The rest were asymptomatic. The use of medication varied among the group.

The introduction is very brief and cites no literature.

The study started November 4, 1999. SO_2 levels weren't available until November 11, 1999. These measurements were made at an alternate monitoring station as the monitoring station initially chosen for the study wasn't available until November 19, 1999.

Some of the PEF data was falsified and excluded from study. There was no way to validate whether the remaining PEF values were reliable.

There was no consideration of a training effect for the Peak flow meter.

There were 18 males and 4 females in the study. The gender distribution in the 16 with valid symptoms is not given.

The symptom scoring system used allowed the asthmatic subject to gauge his or her daily quality of life resulting from asthma, whereas PEF represents a snapshot in time of one physiologic parameter, which may not be representative of the daily severity of asthma.

This study made use of diaries to collect data, followed up with interviews of the subjects and their legal guardians. The age stipulations meant that all were able to complete diaries but were too young to work or drive, limiting occupational exposures and frequent exposures traveling out of the study region. Exclusions to the study were rationalized.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Botter, D.A., Jorgensen, B., Peres, A.A.Q.	**Internal ID:**414
Title: A longitudinal study of mortality and air pollution for Sao Paulo	
Year: 2002	
Abstract: We study the effects of various air-pollution variables on the daily death counts for people over 65 years in Sao Paulo, Brazil, from 1991 to 1993, controlling for meteorological variables. We use a state space model where the air-pollution variables enter via the latent process, and the meteorological variables via the observation equation. The latent process represents the potential mortality due to air pollution, and is estimated by Kalman filter techniques. The effect of air pollution on mortality is found to be a function of the variation in the sulphur dioxide level for the previous 3 days, whereas the other air-pollution variables (total suspended particulates, nitrogen dioxide, carbon monoxide, ozone) are not significant when sulphur dioxide is in the equation. There are significant effects of humidity and up to lag 3 of temperature, and a significant seasonal variation.	
Objective: The main objective of the present investigation is to examine the relationship between air pollution and mortality, controlling for meteorological variable, based on daily death counts for people over 65 years old in Sao Paulo.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
(The figure is difficult to read) Approximate range of SO_2 levels: 5-60μg/m^3.	Study period: 1991 to 1993	Male and female	>65 years	Total death counts not given. Population over 65 years not given.	No info	n/a	n/a

Observations:

"The effect of SO_2, according to our final fitted model, is that a 10 μg/m^3 increase in the SO_2level 3 days ago gives an increase in today's death count of 2.4% (±0.68%).The most likely interpretation is that it is an effect that may reduce the life span of already frail individuals by a few days. Both humidity and lag 3 of temperature are significant." The other air-pollution variables (total suspended particulates, nitrogen dioxide, carbon monoxide, ozone) were not significant when sulphur dioxide is in the equation.
*Statistically significant

Author's Conclusions:

"Our main conclusion is an agreement with other recent findings that there is an association between air pollution and mortality. The results are also in

agreement with a previous study of air pollution and mortality for people over 65 years old in Sao Paulo based on data collected shortly before ours. In studies such as ours involving several air pollution variables, problems with multicollinearity between the variables often make it difficult to assign the air pollution effect to a single variable or combination of variables with any reasonable degree of certainty. However, our analysis unambiguously points to a linear combination of the first few lags of SO_2 as responsible for the effect. The reason for the importance of SO_2 in our study may be related to the particular composition of the air pollution in Sao Paulo, which is mainly generated by vehicle exhausts."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (more info needed) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitoring Was the exposure concentration measured? (+) study period: 1991-1993Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) mortalityWas the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) mortality counts Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (n/a mortality data) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions?

	(n/a mortality data) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+/-) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (more info needed) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (more info needed) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (more info needed) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (no extrapolations made) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The effects of SO_2 levels on the daily death counts for people over 65 years in Sao Paulo, Brazil, from 1991 to 1993, controlling for meteorological variables were analyzed. The effect of air pollution on mortality was found to be a function of the variation in the sulphur dioxide level for the previous 3 days. One possible interpretation of these findings is that it is an effect that may reduce the life span of already frail individuals by a few days. This hypothesis deserves further investigation.

Confidence index:

Even with the faults below, overall this study was very informative as far as the way the results will change depending on the model inputs and manipulation of the model.

There is no information on the monitoring stations, location, etc. There is limited information on the how the mortality data were collected. The details are likely included in previous studies which are referenced, but it is difficult to accurately review the paper when this data is missing.

Excellent detail on statistical model and how the inclusion or exclusion of certain variables will change results. Looks at the results before and after inclusions of dummy variables.

It isn't clear until the discussion, that the authors are conducting this study to compare results from other studies that have analyzed the same data.

The results are presented as model variables, and not RR, OR or confidence intervals, so it is somewhat challenging to read the results and determine significance.

The tables need more explanation of the variables.
The main observation isn't clearly presented in the tables.
This study attempted to control for meteorological variables but did not consider other possible confounders such as demographic data. The subjects involved in the study were not fully characterized; the authors indicated the age group but did not discuss the size of the sample set. Furthermore, no data with regards to the raw air pollution data was provided in tabular form. Graphs provided were difficult to read.
The exposure concentration was not specifically stated in this study. Raw and individual data were not reported, and confidence intervals are not included. However, the modeling is different from previous studies employing meteorological variables and the authors were able to express the effect of air pollution solely in terms of SO_2.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Hong, Y.C., Lee, J.T., Kim, H., Ha, E.H., Schwartz, J., Christiani, D.C.	**Internal ID:** 415

Title: Effects of Air Pollutants on Acute Stroke Mortality

Year: 2002

Abstract: The relationship between stroke and air pollution has not been adequately studied. We conducted a time-series study to examine the evidence of an association between air pollutants and stroke over 4 years (January 1995-December 1998) in Seoul, Korea. We used a generalized additive model to regress daily stroke death counts for each pollutant, controlling for seasonal and long-term trends and meteorologic influences, such as temperature, relative humidity, and barometric pressure. We observed an estimated increase of 1.5% [95% confidence interval (CI), 1.3-1.8%] and 2.9% (95% CI, 0.3-5.5%) in stroke mortality for each interquartile range increase in particulate matter < 10 microm aerodynamic diameter (PM_{10}) and ozone concentrations in the same day. Stroke mortality also increased 3.1% (95% CI, 1.1-5.1%) for nitrogen dioxide, 2.9% (95% CI, 0.8-5.0%) for sulfur dioxide, and 4.1% (95% CI, 1.1-7.2%) for carbon monoxide in a 2-day lag for each interquartile range increase in single-pollutant models. When we examined the associations among PM_{10} levels stratified by the level of gaseous pollutants and vice versa, we found that these pollutants are interactive with respect to their effects on the risk of stroke mortality. We also observed that the effects of PM_{10} on stroke mortality differ significantly in subgroups by age and sex. We conclude that PM_{10} and gaseous pollutants are significant risk factors for acute stroke death and that the elderly and women are more susceptible to the effect of particulate pollutants.

Objective: We used a time series study to examine the association between air pollutants and stroke. Although our main objective was to determine the presence and magnitude of any association between ambient concentrations of PM_{10} and daily stroke mortality in Seoul, we also considered the relative importance of gaseous pollutants with respect to stroke mortality risk.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean: 12.1±7.4 ppb Range: 3.0-46.0 ppb	Jan 1995-Dec 1998	Mean stroke deaths per day: Males-7.3±2.8 Females-8.0±3.0	Mean: <65y-5.0±2.2 >65y10.3±3.5	Average # of stroke deaths: 15.3/d	No info	N/a	n/a

Observations:

Two day lagged concentrations of SO_2 showed the highest risk of stroke mortality. Stroke mortality increased 2.9% (95% CI 0.8-5.0%) for a 5.7ppb increase in SO_2 in the 2 day lag.
Stroke mortality also increased 3.1% (95% CI, 1.1-5.1%) for nitrogen dioxide, and 4.1% for carbon monoxide in a 2-day lag for each interquartile range increase in single-pollutant models. When we examined the associations among PM_{10} levels stratified by the level of gaseous pollutants and vice versa, we found that these pollutants are interactive with respect to their effects on the risk of stroke mortality. We also observed that the effects of PM_{10} on stroke mortality differ significantly in subgroups by age and sex.

An estimated increase of 1.5% in stroke mortality was observed for each interquartile range increase in PM10 aerodynamic diameter and ozone concentrations in the same day.

Author's Conclusions:

In summary, this study demonstrates an association between exposure to air pollutants and stroke mortality. In addition,, we have seen evidence for interactions among air pollutants on the risk of stroke mortality. Our analysis indicates that the elderly and women carry greater risks of stroke mortality due to the effect of particulate pollutants.

We conclude that PM10 and gaseous pollutants are significant risk factors for acute stroke death and that the elderly and women are more susceptible to the effect of particulate pollutants.

"The strongest associations between stroke mortality and PM10 and O3 were seen in the same day, whereas for NO2, SO2, and CO the strongest associations were seen in 2-day lag. Interestingly, the effect sizes of the gaseous pollutants were greater than that of PM10, which is compatible with Moolgavkar's suggestion that gaseous pollutants are more strongly associated with various mortalities including stroke. The estimated risks of stroke mortality for NO2, So2, and CO were much lower and not significant when the same day's concentrations were used instead of concentrations of 2-day lag in the single pollutant analyses.

In summary, this study demonstrates an association between exposure to air pollutants and stroke mortality. In addition, we have seen evidence for interactions among air pollutants on the risk of stroke mortality. Our analysis indicates that the elderly and women carry greater risks of stroke mortality due to the effect of particulate pollutants."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	. (+) Is the number of subjects sufficient to produce statistically significant results? . (-) Are characteristics (*e.g.,* age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? . (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? . (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? . (+-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	. (+) fixed monitors Was the exposure concentration measured? . (+) study period 4 years Is the exposure duration known?
C. Equipment:	. (-) Was a full description of the monitoring devices provided? . (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	. (-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? . (n/a no controls) Were appropriate controls recruited?

	(n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) mortality Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (n/a no symptom analysis) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (significance not reported) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (more info needed) Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (no specific SO_2 conclusions) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The results showed that PM_{10} and gaseous pollutants (including SO_2) may be risk factors of acute stroke mortality, after controlling for confounding factors, such as long-term trends, seasonal changes, day of the week effects and meteorological influence. Pollutant effects are interactive in terms of stroke mortality risk. Authors also report statistically significant modification of stroke mortality risk from the exposure to PM10 by age and sex. It is difficult to determine whether the pollution-related increase of stroke mortality truly represents an increase of stroke mortality or represents only an earlier death, by a few days or weeks, of those already about to die from previous strokes or other cases.

Confidence index:

SO_2 data was collected from 20 automated monitoring stations. More detail is needed on locations of the monitors and variability between the stations.

There are limitations in the exposure assessment.

Good detail on confounders.

Level of significance of results was not reported.

The focus of the paper is on PM_{10} exposure and there is little discussion on SO_2exposure.

This study attempted to control for seasonal and long-term trends, day of the week and meteorologic influences including temperature, relative humidity and barometric pressure. In addition, the author used statistical methods such as inserting autoregressive terms into the model to remove serial correlations of residuals in order to control for any confounding effects caused by omitted time-dependent covariates.

Review – Confidence index ranking:

Low to moderate

CLINICAL REVIEW FORM

Author: Whittenberger, J.L., Frank, R.N. **Internal ID:** 416

Title: Human Exposures to Sulfur Dioxide

Year: 1963

Abstract: An extension of work by Dr. Mary Amdur on guinea pigs in which she found changes in the resistance of the lungs in response to very low concentrations of sulfur dioxide in the range of 2 ppm or less and a remarkable potentiation of these effects when the animals simultaneously inhaled submicron aerosols. This study looks at the effects of sulfur dioxide on humans. Department members volunteered to expose themselves to levels of SO_2 around 1ppm, 5ppm, and 13ppm. After one minute, a definite increase in airway resistance was noticed. This increase progressed for 5 minutes where it plateaued. There was a definite dose response relationship.

Objective: To investigate human exposures to sulfur dioxide.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1, 5, 13 ppm	10 min? (not clearly stated)	No info	No info	No info	No info	n/a	Na/

Observations:

The level of 1ppm in human subjects did not produce significant changes in resistance of the airways except in one subject. During exposure to 5ppm there was an average increase of about 40%; at 13 ppm 72%.

There were no changes in the functional residual capacity.

Within one minute of the initiation of exposure the resistance of the airways increased, and this increase progressed for about five minutes when it reached essentially a plateau. About 50% of the increase occurred within the first minute. After ten minutes there was usually no further change, although sometimes the resistance returned toward normal.

Author's Conclusions:

"We did not observe, as did Sim and Pattle, the development of sensitivity during the course of these exposures which were spread over a period of several months. However, we did have one subject with a history which was suggestive of asthma, at least during childhood. This subject had a higher control resistance and a higher response to SO_2."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	. (not stated) Is the number of subjects sufficient to produce statistically significant results? . (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate

	for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population? **(not stated)** Were subjects informed about the experimental risks? Was this information provided in such as a way as to prevent bias?
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? (-) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(not stated)** Were both nominal and actual exposure concentrations recorded? **(not clearly stated)** Was the duration of exposure appropriate to investigate the primary objective? (-) Was the duration of exposure precisely defined? (-) Was the mode of administration appropriate? **(more info needed)** Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? **(not stated)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Equipment:	(-) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined (-) Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(not stated)** Were the subjects randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(no info)** Was a control group assayed? **(no info)** Is the control regime appropriate? **(no)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(no info)** Was the length of follow-up appropriate? **(no info)** Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(more info needed)** Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(more info needed)** Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject?

	(no info) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(no info) Are the statistical methods used appropriate? (-)Are confidence intervals reported? (no info) Did the investigator appropriately interpret the statistics? (no info) Are the observed trends statistically significant?
G. Interpretations:	(no clear objective)Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (-) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study produced no novel findings. The purpose of this study was not clear and very few details regarding experimental conditions were given.

This study found that SO_2 inhalation increases airway resistance. No change in the functional residual capacity was found except for a slight increase at 13ppm. Respiratory rates and tidal volume are unaffected. The findings in this study, having been done in 1963, are very preliminary.

Confidence index:

This paper was basically a 1 page summary of a study. There was too much information missing to accurately review the study. There are no results presented. The human subjects are described as 'loosely normal'.

This study makes no mention of sample size, or of subject characteristics. It is very unclear if this paper refers to a single study or to any study detailing human exposures to sulfur dioxide. Recruitment of human volunteers, characteristics including age, gender and general health were not given.

Reporting is very vague—study design and conduct are not detailed well.

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM

Author: Strandberg, L.G.	**Internal ID:** 417
Title: SO_2Absorption in the Respiratory Tract	
Year: 1964	

Abstract: A method is presented for studying the absorption of gaseous substances in the breath of rabbits. The method permits differentiated sampling at the maximum of inspiration and expiration. The absorption of SO_2 from approximately 700ppm to 0.05ppm has been studied. The investigation showed that during exposure to low SO_2 concentrations, the relative absorption of SO_2 in the respiratory tract decreased appreciably. The relative content of SO_2 in the trachea during inspiration increased from approximately 5% to about 60%; the corresponding values during expiration, from approximately 2% to approximately.

Objective: The methods used in this study are those presented by Kajland and Strandberg in a preliminary report developed and used for studies of SO_2absorption in rabbits during exposure to concentrations of only a few hundredths of a ppm, with samples of tracheal air taken during maximum inspiration and expiration, ^{35}S labeled SO_2.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
0.05-700ppm SO_2	no info	Rabbit	No info	No info	No info	No info	No info		

Observations:

The absorption of SO_2 in the respiratory tract has been studied from approximately 700ppm to 0.05ppm. Some of the exposures at 200ppm were made with inactive SO_2, and the SO_2 content was determined conductometrically. In the case of exposure to air with high SO_2 contents, the experiments show an absorption in the respiratory tract of approximately 95% at inspiration and approximately 98% at expiration. In the case of low SO_2 contents, on the other hand, considerably less of the SO_2 supplied was absorbed (corresponding values approximately 40% and approx. 80%), and the spread increased between the different experiments. The relation between the SO_2 content of the expired and inspired air, however, does not seem to have changed in the same manner.

Author's Conclusions:

"With the sampling technique described here, the conditions during sampling correspond more or less to the prevailing physiological conditions. The inspiration samples can thus be used in the study of absorption in the upper respiratory tract up until the moment of sampling, while the expiration samples provide evidence of the composition of the alveolar air when the content was lowest."

"The absorption differences found at high and low SO_2 content in these experiments could not be explained, however, by differences in the sampling technique; nor could the large differences in certain cases, be between two experiments at the same concentration level…a probable explanation may, therefore, be changes in the condition of the mucous membrane during exposure to higher SO_2 contents, for example, through an increased secretion of mucus, which is physically capable of dissolving more SO_2 than when there is no irritation to stimulate the secretory glands."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	**(no info)** Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? **(rabbits) no justification** a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* **(no info)** b) Age *Guideline: Adult, i.e.,8-12 weeks.* **(no info)** c) Sex **(no info)** d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* **(no info)** e) Weight variation *Guideline: Below 20% variation* **(no info)** f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	**(-)** Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? **(+)** Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* **(-)** Were the chosen exposure levels appropriate to investigate the primary objective? **(-)** Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(not stated)** Were both nominal and actual exposure concentrations recorded? **(not stated)** Was the duration of exposure appropriate to investigate the primary objective? **(-)** Was the duration of exposure precisely defined? **(n/a)** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the*

	chamber equilibrates. **(+)** Was the mode of administration appropriate? **(no info)** Was the frequency of administration appropriate to investigate the primary objective? **(-)**Was evidence provided to justify the dosing regime? **(not stated)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(not stated)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(n/a)** Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* **(no info)** Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* **(not stated)** Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* **(no info)** Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(+)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(-)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(no info)** Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* **(no)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided?

	(no info) Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.,* information pertaining to the mechanism used to generate random assignment) **(not stated)** Was a control group assayed? **(no info)** Is the control regime appropriate? **(no info)** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? **(more info needed)** Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	**no info** Are the statistical methods used appropriate? (-) Are confidence intervals reported? **(no interpretation)** Did the investigator appropriately interpret the statistics? **(no info)** Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? **(more detail needed)** Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? **(no clear conclusions)** Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal?

	(no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study describes a sampling technique with conditions during sampling corresponding to physiological conditions. The authors suggest that variation in sampling techniques could explain discrepancies in the literature concerning the absorption of SO_2.

Results from this study show that there is a discrepancy between high concentration absorption of SO_2 in the respiratory tract of rabbits and low concentration absorption. To this, the report gives two possible explanations: at low concentrations, the SO_2 may dissolve relatively slowly in water, or changing conditions of the mucous membrane during exposure to higher concentrations.

Confidence index:

This study lacks significant detail in all areas. The information provided for test animals, housing/feeding, exposure conditions, equipment, procedural, data collection, and data analysis. There is no information on the dosing regime and the exposure duration.

The test animals are not described. There is no justification given for the methods used. Also, it is unclear whether a control group was assayed. Therefore, it is not clear whether confounding variables were limited. The presentation of the results is inadequate. Though this study was very descriptive on its methods, it is not clear whether the results are statistically significant as the number of rabbits in the study was not stated.

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM

Author: Balchum, O.J., Dybicki, J., Neneely, G.R.	**Internal ID:** 418
Title: Absorption and distribution of $S^{35}O_2$ inhaled through the nose and mouth by dogs.	
Year: 1959	
Abstract: Dogs breathing $S^{35}O_2$ through the nose and mouth retained a smaller proportion of the inhaled gas in the trachea, lungs, hilar lymph nodes, and liver and spleen than those breathing similar concentrations via a tracheostomy. This conclusion is substantiated by tissue S^{35} concentration assays and measurements of the compliance of the lungs and thorax and of pulmonary resistance. The upper airways therefore partially protect the lungs from exposure to sulfur dioxide.	
Objective: The following study was designed: a) to evaluate the role of the upper airways as a protective mechanism, and to detect whether they are effective in reducing the amount of SO_2gaining access to the lungs and being absorbed by them, and b) to determine quantitatively the distribution of S^{35} to various organs and tissues of the dog under these conditions of exposure.	

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
1.1-141ppm (varied per dog)	20-40 minutes (varied per dog)	Dogs	No info	No info	No info	12	No info	n/a	n/a

Observations:

There were no statistically significant results. Each dog inhaled a different concentration of $SO_2$and therefore, for each concentration, n=1. Also the exposure duration was different for each dog. However, the following results were cited in the published study: There was an average decrease in the compliance of the lungs and thorax. There was a much smaller percentage of the retained ^{35}S present in the trachea and lungs of the animals breathing ^{35}S through the mouth and nose than in animal breathing gas via a tracheostomy. The liver and spleen of mouth and nose breathing dogs contained a lesser proportion of the retained ^{35}S than did the corresponding organs of animals breathing this gas via a tracheostomy. The concentrations of ^{35}S in the trachea and lungs were much lower in the present group than in dogs breathing $^{35}SO_2$ in the trachea and lungs and were much lower in the present group than in dogs breathing $^{35}SO_2$ via a tracheostomy. Comparison with animals retaining approximately the same total amounts of ^{35}S indicated that the concentrations in the trachea, lungs, hilar lymph nodes, liver and spleen were strikingly lower in animals breathing $^{35}SO_2$ via the nose and mouth than in those breathing via a tracheostomy. The ^{35}S blood levels in two animals were one-half, and in the remaining three instances many times less in nose- and mouth breathing dogs.

Author's Conclusions:

"Dogs breathing $S^{35}O_2$ through the nose and mouth retained a smaller proportion of the inhaled gas in the trachea, lungs, hilar lymph nodes, and liver and spleen

than those breathing similar concentrations via a tracheostomy. This conclusion is substantiated by tissue S^{35} concentration assays and measurements of the compliance of the lungs and thorax and of pulmonary resistance. The upper airways therefore partially protect the lungs from exposure to sulfur dioxide."

"The decreases in compliance were most likely associated with an increased pulmonary resistance, as no frank edema fluid, mucus, or froth was visible in the bronchi and lungs at autopsy. It is possible that the compliance changes were as a result of pulmonary vascular engorgement.
It has been shown that the mucous membrane of the trachea may absorb $^{35}SO_2$ when it alone is exposed, but at a reduced rate, and the same conditions may apply to the mucosa of the upper airways. Since the retention of ^{35}S by the liver and spleen was less than in tracheostomy-breathing animals, it is likely that not enough additional time has elapsed to allow $^{35}SO_2$ absorption from the upper airways into the bloodstream with subsequent uptake by the liver and spleen.
The tracheal, lung and hilar lymph node concentrations of ^{35}S were lower in mouth and nose-breathing than in tracheostomy-breathing dogs. These data substantiate the conclusion based on organ retention estimates that the upper airways aid in partially preventing access of SO_2 in the trachea and lungs."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	(-) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? **(dog) not justified** a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* **(no info)** b) Age *Guideline: Adult, i.e.,8-12 weeks.* **(no info)** c) Sex d) Pre-test health **(no info)** *Guideline: Females should be nulliparous and non-pregnant.* e) Weight variation (-) >20% *Guideline: Below 20% variation* f) Source **(-) no info** *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.*

	(-) Were the chosen exposure levels appropriate to investigate the primary objective? **(-)** Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(not stated)** Were both nominal and actual exposure concentrations recorded? **(-)** Was the duration of exposure appropriate to investigate the primary objective? **(-)** Was the duration of exposure precisely defined? **(not stated)** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(more info needed)** Was the mode of administration appropriate? **(more info needed)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(not stated)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(not stated)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(no info)** Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* **(no info)** Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* **(no info)** Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* **(no info)** Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*

D. Equipment:	**(more detail needed)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(-)** Was a full description of the monitoring devices provided? **(not stated)(** What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(not stated)** Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* **(no)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(no info)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(subjects served as own controls)** Was a control group assayed? **(more detail needed)** Is the control regime appropriate? **(-)** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	**(more info needed)** Were appropriate endpoints examined? (-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (not stated) Did qualified personnel conduct the exams? **(more info needed)** Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each test animal?
G. Data analysis:	**(more info needed)** Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(more info needed)** Did the investigator appropriately interpret the statistics?

	(-) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables? (-) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no statistically significant results. Each dog inhaled a different concentration of $SO_2$and therefore, for each concentration, n=1. Also the exposure duration was different for each dog. Therefore, it is impossible to make group comparisons.

The findings were not clinically or toxicologically significant.

Confidence index:

The study is lacking significant information in all areas of the study.

There is no information on the test animals (type, age, gender), exposure conditions, housing and feeding and equipment.

Each dog is exposed to a different concentration of $SO_2$for a different exposure duration (20 to 40 min). The authors report results for the whole group, which is inconsistent given that the exposure conditions were different for each dog.

The weight variation of the dogs was approximately 30 percent.

The study does not make use of statistical methods, and does not clearly identify the reagents used in the procedure.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Schwartz, J., Ballester, F. Saez, M., Perez-Hoyos, S., Bellido, J., Cambra, K., Arribas, F., Canada, A., Perez-Boillos, J.J., Sunyer, J.	**Internal ID:** 419

Title: The Concentration – Response Relation between Air Pollution and Daily Deaths

Year: 2001

Abstract: Studies on three continents have reported associations between various measures of airborne particles and daily deaths. Sulfur dioxide has also been associated with daily deaths, particularly in Europe. Questions remain about the shape of those associations, particularly whether there are thresholds at low levels. We examined the association of daily concentrations of black smoke and SO_2 with daily deaths in eight Spanish cities (Barcelona, Bilbao, Castellon, Gijon, Oviedo, Valencia, Vitoria, and Zaragoza) with different climates and different environmental and social characteristics. We used nonparametric smoothing to estimate the shape of the concentration-response curve in each city and combined those results using a metasmoothing technique developed by Schwartz and Zanobetti. We extended their method to incorporate random variance components. Black smoke had a nearly linear association with daily deaths, with no evidence of a threshold. A 10 $\mu g/m^3$ increase in black smoke was associated with a 0.88% increase in daily deaths (95% confidence interval, 0.56%-1.20%). SO_2 had a less plausible association: Daily deaths increased at very low concentrations, but leveled off and then decreased at higher concentrations. These findings held in both one- and two-pollutant models and held whether we optimized our weather and seasonal model in each city or used the same smoothing parameters in each city. We conclude that the association with particle levels is more convincing than for SO_2, and without a threshold. Linear models provide an adequate estimation of the effect of particulate air pollution on mortality at low to moderate concentrations.

Objective: Here we report results of analyses in either cities in Spain, examining the concentration-response relation between daily deaths and both SO_2 and airborne particles.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
11-44$\mu g/m^3$	1990-1996 (years of study varied per city)	No info	No info		No info	n/a	Na/

Observations:

When air pollution was assumed to have a linear concentration response relationship with daily deaths, SO_2 showed weak evidence of an association with mortality in these cities (0.27% increase in daily deaths for a 10$\mu g/m^3$ in exposure, 95% CI 0.18-0.73%.

For SO_2, the risk increases until SO_2 concentrations of about 20-30$\mu g/m^3$ and then levels out and begins to decline with further increases in concentration. The same 50 $\mu g/m^3$ increase from the baseline is associated with only a 0.5% increase in daily deaths, which is only 40% of what would have been predicted based on fitting a linear concentration response relation.

Author's Conclusions:

"We conclude that the association with particle levels is more convincing than for SO_2, and without a threshold. Linear models provide an adequate estimation of the effect of particulate air pollution on mortality at low to moderate concentrations."
"The results for SO_2 are less supportive of a causal association. The smooth plot does not suggest a consistent increase in daily deaths with exposure, and the confidence bands for a linear fit include no effect. In the two-pollutant models, the results are even more convincingly null."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no control) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (not stated) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (n/a) Was the timing and length of follow-up appropriate? (n/a) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (n/a) Was the nature and severity of symptoms graded against an explicitly defined scale? ((n/a)) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions?

	(some) Was individual data reported for each subject? (n/a) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+/-) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The association between $SO_2$and mortality in the eight cities was weak and inconsistent. The authors claim that the results are less supportive of a causal association. However, this study would not have been able to determine causality.

One of the main conclusions from this study is that, in Europe, SO_2 may act as a surrogate for another pollutant, probably airborne particles. This would explain the lack of cause/effect relationship between SO_2 and daily mortality. This conclusion has been reported in other articles.

Confidence index:

Standard methodology used.

The years of study for the cities differed as did the populations.

The quality of the results depends on the quality of the individual study in each city.

The characteristics of each city were very different, and confounders may not have been adequately controlled.

The relationship between SO_2 and mortality was assumed to be linear.

Although this study was thorough in its interpretations, it lacked in reporting the methods. Little is known about equipment for measurement and procedure and there is no information on test subjects. Observed trends are statistically significant assuming that the whole population was observed.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Wong, T.W., Tam, W.S., Yu, T.S., Wong, A.H.S.

Internal ID: 422

Title: Associations between daily mortalities from respiratory and cardiovascular diseases and air pollution in Hong Kong, China

Year: 2002

Abstract: OBJECTIVE: To investigate the association between ambient concentrations of air pollutants and respiratory and cardiovascular mortalities in Hong Kong. METHODS: Retrospective ecological study. A Poisson regression of concentrations of daily air pollutants on daily mortalities for respiratory and cardiovascular diseases in Hong Kong from 1995 to the end of 1998 was performed using the air pollution and health: the European approach (APHEA) protocol. The effects of time trend, seasonal variations, temperature, and humidity were adjusted. Autocorrelation and overdispersion were corrected. Daily concentrations of nitrogen dioxide (NO_2), sulphur dioxide (SO_2), ozone (O_3), and particulate matter <10 microm in aerodynamic diameter (PM_{10}) were averaged from eight monitoring stations in Hong Kong. Relative risks (RRs) of respiratory and cardiovascular mortalities (per 10 μg/m3 increase in air pollutant concentration) were calculated. RESULTS: Significant associations were found between mortalities for all respiratory diseases and ischaemic heart diseases (IHD) and the concentrations of all pollutants when analysed singly. The RRs for all respiratory mortalities (for a 10 $\mu g/m^3$ increase in the concentration of a pollutant) ranged from 1.008 (for PM_{10}) to 1.015 (for SO_2) and were higher for chronic obstructive pulmonary diseases (COPD) with all pollutants except SO_2, ranging from 1.017 (for PM_{10}) to 1.034 (for O_3). RRs for IHD ranged from 1.009 (for O_3) to 1.028 (for SO_2). In a multipollutant model, O_3 and SO_2 were significantly associated with all respiratory mortalities, whereas NO_2 was associated with mortality from IHD. No interactions were detected between any of the pollutants or with the winter season. A dose-response effect was evident for all air pollutants. Harvesting was not found in the short term. CONCLUSIONS: Mortality risks were detected at current ambient concentrations of air pollutants. The associations with the particulates and some gaseous pollutants when analysed singly were consistent with many reported in temperate countries. PM_{10} was not associated with respiratory or cardiovascular mortalities in multipollutant analyses.

Objective: To investigate the association between ambient concentrations of air pollutants and respiratory and cardiovascular mortalities in Hong Kong.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Mean: $16.68 \pm 11.59 \mu g/m^3$	1995-1998	No info	No info	Deaths from all respiratory diseases: 17 ± 5/day	No info	n/a	n/a

Observations:

Relative risks were calculated for a $10 \mu g/m^3$ increase in SO_2. There was a significant increase in mortality for respiratory disease (RR 1.015 95% CI 1.001-1.029) For pneumonia and influenza, the RRs were significant (1.02 95% CI 1.003-1.039). The results were also significant for Ischaemic heart disease (RR 1.028 95% CI 1.01-1.044).

The RRs for all respiratory mortalities ranged from 1.008 (for PM_{10}) to 1.015 (for SO_2) and were higher for chronic obstructive pulmonary diseases with all pollutants except SO_2, ranging from 1.017 (for PM_{10}) to 1.034 (for O_3). RRs for IHD ranged from 1.009 to 1.028 (for SO_2).

A dose-response effect was evident for all air pollutants. Harvesting was not found in the short term.

During the 4 years of the study, 46% of the deaths were caused by respiratory and circulatory diseases. The relative risks of NO_2 and SO_2 were significant for pneumonia and influenza. No significant interaction between any pair of pollutants was found in the pair-wise analyses or between any pollutant and the cold season. When all pollutants were included in the models, ozone and SO_2 remained significantly associated with respiratory mortalities, whereas NO_2 was associated with mortality from ischaemic heart disease.

Author's Conclusions:

"Mortality risks were detected at current ambient concentrations of air pollutants. The associations with the particulates and some gaseous pollutants when analysed singly were consistent with many reported in temperate countries. PM_{10} was not associated with respiratory or cardiovascular mortalities in multipollutant analyses."

"We found significant associations of all four air pollutants with mortalities from respiratory disease and IHD, the effect size being strongest for chronic obstructive pulmonary disease. Although the multi-pollutant model selected SO_2 and O_3 as significant pollutants for respiratory deaths and NO_2 for deaths from ischaemic heart disease, other pollutants such as PM_{10} might play an important part. The joint effects of the air pollutants are still poorly understood and comparative studies in cities with different pollutant profiles are warranted."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) **Hong Kong** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **fixed monitors** Was the exposure concentration measured? (+) **1995-1998**Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls?

	(+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) **mortality** Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (+) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? **(No extrapolations outside study population)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

In this study, barely significant associations were found with respiratory mortalities and ischaemic heart diseases and ambient concentrations for SO_2, PM_{10}, NO_2, and O_3. The magnitude of the effects were quite small. The confidence intervals for the association between respiratory mortality and SO_2 include RR=1.
A dose response relation between the relative risks and the concentrations of the pollutants was found for all four air pollutants.

Confidence index:

Several non-significant or barely significant results reported as significant
Standard methodology used.
The harvesting effect was investigated.
The locations of the monitoring stations is not provided.
The correlation / variability between stations is not reported.
Consistency in results reported in other studies.
Due to the large sample size (essentially the population of Hong Kong), it was impossible to accurately state characteristics of all subjects involved. In addition, no control group was assayed for this study nor was type of blinding indicated. However, statistical methods used were appropriate for data analysis, in accordance with the European approach (APHEA) protocol to air pollution and health.

Review – Confidence index ranking:

Low to moderate

DRAFT

CASE-CONTROL REVIEW FORM

Author: Wong, C.M., Atkinson, R.W., Anderson, H.R., Hedley, A.J., Ma, S., Chau, P.Y.K., Lam, T.H.	**Internal ID:** 423
Title: A Tale of Two Cities: Effects of Air Pollution on Hospital Admissions in Hong Kong and London Compared	
Year: 2002	

Abstract: The causal interpretation of reported associations between daily air pollution and daily admissions requires consideration of residual confounding, correlation between pollutants, and effect modification. If results obtained in Hong Kong and London--which differ in climate, lifestyle, and many other respects--were similar, a causal association would be supported. We used identical statistical methods for the analysis in each city. Associations between daily admissions and pollutant levels were estimated using Poisson regression. Nonparametric smoothing methods were used to model seasonality and the nonlinear dependence of admissions on temperature, humidity, and influenza admissions. For respiratory admissions (> or = 65 years of age), significant positive associations were observed with particulate matter < 10 microm in aerodynamic diameter (PM_{10}), nitrogen dioxide, sulfur dioxide, and ozone in both cities. These associations tended to be stronger at shorter lags in Hong Kong and at longer lags in London. Associations were stronger in the cool season in Hong Kong and in the warm season in London, periods during which levels of humidity are at their lowest in each city. For cardiac admissions (all ages) in both cities, significant positive associations were observed for PM_{10}, NO_2, and SO_2 with similar lag patterns. Associations tended to be stronger in the cool season. The associations with NO_2 and SO_2 were the most robust in two-pollutant models. Patterns of association for pollutants with ischemic heart disease were similar in the two cities. The associations between O_3 and cardiac admissions were negative in London but positive in Hong Kong. We conclude that air pollution has remarkably similar associations with daily cardiorespiratory admissions in both cities, in spite of considerable differences between cities in social, lifestyle, and environmental factors. The results strengthen the argument that air pollution causes detrimental short-term health effects.

Objective: We have conducted parallel analyses of the short-term associations between air pollution and daily hospital admissions in Hong Kong and London to compare and contrast the health effects of air pollution in the two cities.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Hong Kong:17.7±12.3μg/m^3 London:23.7±12.3μg/m^3	Hong Kong:1995-1997 London:1992-1994	No info	All ages	Average admissions per day for asthma, respiratory ,cardiac, IHD: Hong Kong:7.8,91.3, 98.7,36.0 respectively London:14.1,58.3,121.1,51.3	No info	n/a	Na/

Observations:

No statistically significant associations between asthma admissions and SO_2 (10μg/m^3) in either of the cities. For respiratory admissions, there were small, positive, and statistically significant associations with SO_2 in Hong Kong. For cardiac diseases, both cities showed significant positive associations of comparable size with SO_2.

For respiratory admissions, significant positive associations were observed with particulate matter < 10µm in aerodynamic diameter (PM_{10}), nitrogen dioxide, sulfur dioxide, and ozone in both cities. These associations tended to be stronger at shorter lags in HK and at longer lags in London. They were stronger in the cool season in HK and in the warm season in London, periods during which levels of humidity are at their lowest in each city. For cardiac admissions in both cities, significant positive associations were observed for PM_{10}, NO_2, and SO_2 with similar lag patterns. Associations tended to be stronger in the cool season. The associations with NO_2 and SO_2 were the most robust in two-pollutant models. Patterns of association for pollutants with ischemic heart disease were similar in the two cities.

Author's Conclusions:

We conclude overall that there are considerable similarities in the effects of each pollutant, although the explanations for some differences, including a lack of association with asthma admissions in adults in Hong Kong, difference in the lag pattern for all respiratory admissions and opposite directions of effect for O_3 and cardiac admissions, remain uncertain. Overall, we consider the similarities to outweigh the differences; thus, we conclude that our study strengthens the argument for the causality of air pollution associations with hospital admissions."

"We conclude that air pollution has remarkably similar associations with daily cardiorespiratory admission in both cities, in spite of considerable differences between cities in social, lifestyle, and environmental factors. The results strengthen the argument that air pollution causes detrimental short-term health effects."

"We observed that the lag patterns for respiratory mortality associated with NO_2, PM_{10}, and SO_2 also followed the respective patterns for respiratory admissions. The other difference in the effects of NO_2, PM_{10}, and SO_2 was that in Hong Kong, the effects were stronger in the cool season, whereas they were stronger in the warm season in London. One common factor here is that the humidity is lower in the season showing the largest effects. It may also be relevant that the average levels of all pollutants apart from SO_2 are highest during the cool season in Hong Kong."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(probably not) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (not stated) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval?

	(n/a no controls) Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(n/a)** Was the timing and length of follow-up appropriate? **(n/a)** Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a)** Was the nature and severity of symptoms graded against an explicitly defined scale? (no info) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (some) for each city Was individual data reported for each subject? **(n/a)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(few)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-)Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (more info needed) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (no specific extrap. For SO2 Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study attempted to determine if the effects of air pollution on daily hospital admissions are consistent between Hong Kong and London. The author questions whether the associations are consistent in widely varying environments but concludes that the similarities between the two centers outweigh the differences and recommends the use of many cities for future studies in order to learn more about the effects of different pollution mixtures.

The findings for SO_2 and mortality were inconsistent between the two cities. The results for SO_2 were not significant.

SO_2 and NO_2 associations were the most robust in two-pollutant models. It was concluded that air pollution has remarkably similar associations with daily cardio-respiratory admissions regardless of the city.

Confidence index:

There is no information on the number of monitoring stations and their location.

Standard methodology used.

The cities were very different in climate, population, number of deaths per each cause and lifestyle.

The samples sizes were likely too small to have statistical power.

The clinical diagnoses could not be validated.

This study attempted to summarize demographic data from each population, and used Poisoon regression to model seasonal, long-term trends and weather factors, minimizing confounding factors. Identical statistical methods were used for analysis in each city.

Review – Confidence index ranking:

Moderate to low

CASE-CONTROL REVIEW FORM

Author: Xu, X., Li, B., Huang, H.	**Internal ID:** 424
Title: Air Pollution and Unscheduled Hospital Outpatient and Emergency Room Visits	
Year: 1995	
Abstract: We conducted a time-series analysis of daily hospital visits and air pollution data to assess acute effects of air pollution on daily unscheduled outpatient visits to internal medicine, pediatric, and emergency departments in the No. 3 Affiliated Hospital of Beijing Medical University in Beijing, China. Sulfur dioxide was marginally significantly associated with total outpatient visits (beta = 41.5, SE = 24.2) and significantly associated with internal medicine (beta = 14.6, SE = 6.7), pediatric (beta = 12.7, SE = 3.7), and emergency room visits (beta = 6.8, SE = 2.7). Total suspended particulates (TSP) was a significant predictor for total outpatient (beta = 21.1, SE = 7.7) and pediatric visits (beta = 3.4, SE = 1.3) and a marginally significant predictor of internal medicine visits (beta = 4.2, SE = 2.2). In a season-specific analysis, SO_2 was a significant predictor for total hospital outpatient visits in summer, although the mean daily SO_2 concentration was only 17 μg/m^3 (maximum = 51 μg/m^3). In winter, SO_2 was significantly associated with internal medicine, pediatric, and emergency room visits, and TSP was associated with total outpatient visits. This study suggests an exposure-response relationship between TSP and SO_2 and hospital outpatient visits, both at high air pollution levels and at levels well below air quality standards recommended by the World Health Organization.	
Objective: Data on daily outpatient and emergency room visits collected from the No. 3 Affiliated Hospital of Beijing Medical University in Beijing provide an opportunity to examine the acute effects of air pollution on hospital outpatient visits.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Mean 118±133μg/m^3 Range: 6-478μg/m^3	Study period: 1 year	Male and female	No info	Average daily hospital outpatient visits= 1836 Average daily emergency room visits=182	No info	n/a	n/a

Observations:

Models including SO_2 and TSP were fit separately. Air pollution effects were expressed as the increase in the number of outpatient or emergency room visits for each 100 $\mu g/m^3$ increase in SO_2 or TSP. SO_2 and TSP concentrations were much lower in the summer than in the winter. Of note, the mean daily SO_2 concentration was only 17 $\mu g/m^3$ (maximum 51 $\mu g/m^3$) during the summer.
For a 100 $\mu g/m^3$ increase in SO_2, SO_2 was significantly associated with internal medicine and pediatric outpatient visits and emergency room visits, and marginally significantly associated with total outpatient visits.
SO_2 was a significant predictor for total hospital outpatient visits in the summer and for internal medicine and pediatric outpatient visits and emergency room visits in the winter.

Total suspended particulates was a significant predictor for total outpatient and pediatric visits and a marginally significant predictor of internal medicine visits..
A season specific regression analysis was performed, with adjustment for temperature, humidity, and day of the week. SO_2 was a significant predictor for total hospital outpatient visits in the summer and for internal medicine and pediatric outpatient visits and emergency room visits in the winter.

Author's Conclusions:

"This study suggests an exposure-response relationship between TSP and SO_2 and hospital outpatient visits, both at high air pollution levels and at levels well below air quality standard recommended by the World Health Organization."
"A statistically significant relation was found between increased levels of SO_2 and daily hospital internal medicine and pediatric outpatient visits, but not with total outpatient visits. One explanation for this result is that internal medicine and pediatric outpatient visits consisted of a large proportion of respiratory diseases, which may be more closely associated with increases in air pollution than the other diseases. The association of SO_2 with internal medicine, pediatric outpatient, and emergency room visits was significant only in the winter, not in the summer, again suggesting that increased hospital visits associated with air pollution may be due to respiratory diseases. A similar trend was also noticed for TP. Unfortunately, the information on disease-specific outpatient visits was not available for this analysis."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed0** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **fixed monitors** Was the exposure concentration measured? (+) **study period 1 year**Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided?

	(no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? **(n/a hospital admissions)** Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) **hospital admissions** Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable? (-) Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)**Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

$SO_2$was not significantly associated with total outpatient visits and significantly associated with internal medicine, pediatric and emergency room visits. The results of this study suggest an exposure-response relationship between $SO_2$and hospital visits at high and low concentrations of SO_2. When stratified by season, the results were inconsistent; significance was reported with total patient visits in the winter only. In the summer significance was reported for pediatric and internal visits. Disease specific analysis may help to explain these results.

A strength of this study is that, while other studies have used data from weekly visits, this study collects data from daily visits. Because this study measured air pollution for about 2-3 weeks each month, days on which air samples were collected were selected at random, independently of air pollution levels.

There is an exposure-response relationship between TSP and SO_2 and hospital outpatient visits both at high air pollution levels and at levels well below air quality standards recommended by WHO.

Confidence index:

Further information is needed on the location of the monitoring stations with respect to the hospital and the patient's residence.

Only patients who are able to pay the registration fee will go to the hospital.

More detail is needed on the statistical methods. RR and confidence intervals were not reported.

Because air samples were collected and analyzed for about 2-3 weeks of each month at each monitoring station, the daily air pollution data were not complete. However, the authors state that the days on which air samples are collected are selected at random are independent of air pollution levels.

The previous studies in Beijing, which the authors report are consistent with the results of this study, were all conducted by the same group as this investigation.

The present study takes advantage of China's medical system, where patients seek medical care in their designated local hospitals, and therefore accounts for all doctor visits.

This study utilized subjects from an area with few industries and where bicycles are the main form of transportation. The coal stove is the major air pollution source. The author further specified which days of the year that the subjects would not have been able to visit doctors in the region.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Tenias, J.M., Ballester, F., Rivera, M.L.	**Internal ID:** 425

Title: Association between hospital emergency visits for asthma and air pollution in Valencia, Spain

Year: 1998

Abstract: OBJECTIVE: To assess the short term effect of concentrations of black smoke, sulphur dioxide (SO_2), nitrogen dioxide (NO_2) and ozone (O_3) in ambient air on emergency room visits for asthma in the city of Valencia, Spain during the period 1994-5. METHODS: Ecological study with time series data and application of Poisson regression. Associations between number of daily emergency visits in a city's hospital and concentrations of air pollutants were analyzed taking into account potential confounding factors by the standardized protocol of the air pollution and health: a European approach (APHEA) project. RESULTS: Mean (range) daily number of emergency room visits for asthma was 1 (0-5). Concentrations of all pollutants studied remained within current air quality standards. The association between an increase of 10 $\mu g/m^3$ in ambient air pollution and asthma, measured as a relative risk (RR) of emergency visits, was significant for NO_2 24 hour mean (lag 0, RR 1.076, 95% confidence interval (95% CI) 1.020 to 1.134), NO_2 hour maximum (lag 0, RR 1.037, 95% CI 1.008 to 1.066), and O_3 hour maximum (lag 1, RR 1.063, CI 95% 1.014 to 1.114). The association was not significant for SO_2 or for black smoke during the period analyzed. The effects were not significantly different for the time of year, cold months (November to April), or warm months (May to October). CONCLUSIONS: Current concentrations of ambient air pollution in Valencia are significantly associated with emergency room visits for asthma. This association is high and more consistent for NO_2 and O_3 than for particulate matter and SO_2 (classic pollutants).

Objective: The objective of this study was to assess the relation between daily concentrations of ambient air pollution and the number of emergency visits for asthma in a hospital in the city of Valencia.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
24 hr Mean: $26.6\mu g/m^3$ 1hr mean: $56.3\mu g/m^3$	1994-1995	No info	No info	734 cases of asthma	No info	n/a	n/a

Observations:

There was an average of 734 asthma cases, 1 per day and a maximum of five per day. Sulfur dioxide had a positive, but not significant, association. The relative risks were calculated for a 10 $\mu g/m^3$ increase in $SO_2$and all of the 95% CI included 1.

Concentrations of all pollutants studied remained within current air quality standards. The association between an increase of 10 $\mu g/m^3$ in ambient air pollution and asthma, measured as a relative risk of emergency visits, was significant for NO_2 24 hour mean (lag 0, RR 1.076, 95% confidence interval (95% CI) 1.020 to 1.134), NO_2 hour maximum (lag 0, RR 1.037, 95% CI 1.008 to 1.066) and O_3 hour maximum (lag 1, RR 1.063, CI 95% 1.014 to 1.114). The effects were not significantly different for the time of year, cold months (November to April), or warm months (May to October).

*Statistically significant

Author's Conclusions:

"In conclusion, the findings of this study show a positive relation between concentrations of ambient pollution and emergency visits for asthma, even by the standards considered as safe. This could be relevant to public health policy due to the number of people exposed, the existence of high risk groups, and the possibility of introducing control measures."

"In this study the association between asthma visits was high and more consistent for NO_2 and O_3 than for particulate matter and SO_2. Furthermore all pollutants studied, except smoke, showed a greater effect in warm months than in cold ones. In general, the concentrations of pollutants were moderate or even low for SO_2, and O_3. This could partly explain the non-significant association when we carried out the analyses for the periods specific to these pollutants. It has been suggested that the greater effect of the pollutants during the warmer months could be a result of higher exposure based on modified activity patterns."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitors Was the exposure concentration measured? (+) study period 2 years Is the exposure duration known?
C. Equipment:	(+) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed?

	(more info needed) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(no)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable? (+) Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(no specific SO_2 conclusion)** Do the conclusions make biological sense? **(no extrapolations re SO_2 made)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no significant associations between SO_2 and hospital emergency visits due to asthma. This may have been due to the relatively small number of asthma visits or low concentrations of SO_2.

Findings of the study suggest a relationship between concentrations of ambient pollution and emergency visits for asthma.

Confidence index:

Standard methodology was used – from the APHEA project.

The hospital serviced approximately 200,000 people. The total population was approximately 750,000. More information is needed to determine if this group is representative of the general population.

There is some detail on reasons of exclusions of certain cases.

The method of data collection had been validated and widely documented.

Asthma cases were identified from diagnosis records by trained nurses. There is still the possibility of misdiagnosis as diagnosis could only be interpreted from past records. ICD codes were not used to make diagnosis of asthma.
SO_2levels were collected from 14 manual and five automatic stations. The data from the automatic network was not available for 1993 and only part of 1994. The variability in the measurements between stations is not provided.
Even though a significant association was not observed for SO_2 the authors still provided a somewhat detailed discussion on SO_2 results in other studies.
This study attempted to control for seasonal and long-term trends, day of the week and meteorologic influences including temperature, relative humidity and barometric pressure. In addition, the author used statistical methods such as inserting autoregressive terms into the model to remove serial correlations of residuals in order to control for any confounding effects caused by omitted time-dependent covariates.
The authors point out that a limitation of this study is the measurement of exposure, which prevents extrapolating the conclusions to an individual level. The authors also point to another limitation being the relatively small size of the population.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Schwartz, J., Dockery, D.W., Neas, L.M., Wypij, D., Ware, J.H., Spengler, J.D., Koutrakis, P., Speizer, F.E., Ferris, B.G.	**Internal ID:** 426

Title: Acute Effects of Summer Air Pollution on Respiratory Symptom Reporting in Children

Year: 1994

Abstract: A daily diary of respiratory symptoms was collected from the parents of 1,844 school children in six U.S. cities to study the association between ambient air pollution exposures and respiratory illness. A cohort of approximately 300 elementary school children in each of six communities were asked to keep a daily log of the study child's respiratory symptoms for one year. Daily measurements of ambient sulfur dioxide, nitrogen dioxide, ozone, inhalable particles (PM_{10}), respirable particles ($PM_{2.5}$), light scattering, and sulfate particles were made, along with integrated 24-h measures of aerosol strong acidity. The analyses were limited to the five warm season months between April and August. Significant associations were found between incidence of coughing symptoms and incidence of lower respiratory symptoms and PM_{10}, and a marginally significant association between upper respiratory symptoms and PM_{10}. There was no evidence that other measures of particulate pollution including aerosol acidity were preferable to PM_{10} in predicting incidence of respiratory symptoms. Significant associations in single pollutant models were also found between sulfur dioxide or ozone and incidence of cough, and between sulfur dioxide and incidence of lower respiratory symptoms. A change in 24-h PM_{10} concentration from 20-50 $\mu g/m^3$ was associated with a relative odds of 1.53 (95% CI 1.20-1.95) for the incidence of lower respiratory symptoms, a relative odds of 1.22 (95% CI 1.03-1.45) for the incidence of coughing, and a relative odds of 1.22 (95% CI 0.98-1.52) for the incidence of upper respiratory symptoms. A change in 24h ozone concentration of 30 ppb was associated wit a relative odds of 1.22 (95% CI 0.96—1.49) for the incidence of coughing. The relationship between ozone and incidence of cough was independent of other pollutants. However, the relationship between sulfur dioxide and incidence of lower respiratory symptoms appeared to drive only from a few influential observations, and could be confounded by PM_{10}. Aerosol acidity was no associated with the incidence of any respiratory symptom. The highest daily PM_{10} concentration was $117 \mu g/m^3$ during the study, indicating that these relationships occurred at concentrations below the ambient air quality standard.

Objective: This report presents results from a study of the association between respiratory symptoms in a sample of elementary school children in six U.S. cities and daily air pollution measurements including aerosol acidity measurements.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Maximal 24hr average: 82ppb	Study period: 1 year	Male and female	Grades 2-5	1844	No info	n/a	>22ppb for lower respiratory symptoms (See below in Observations)

Observations:

Cough incidence was not significantly associated with SO_2 (OR for 10ppb=0.99, 95% CI = 0.90-1.10). For longer average times, the estimated effect of the average concentration over the previous 4 d was statistically significant for SO_2 (OR for 10ppb=1.16, 95%CI=1.02-1.32). The local nonparametric smooth showed increased cough incidence only above a 4-d mean of 20 ppb, that is, less than 5% of the data. The test for nonlinearity was significant (p=0.002). An analysis testing for an association with 4d mean SO_2 restricted to days below 20 ppb was far from statistical significance (p=0.54). Thus, at the relatively low SO_2 and NO_2 ambient concentrations observed in this study, no clear associations with cough incidence could be observed.

There was no significant serial correlation in the incidence of lower respiratory symptoms. Sulfur dioxide was associated with incidence of lower respiratory symptoms (OR for 10 ppb = 1.28, 95% CI 1.13-1.46, p=0.0011). No increase in the incidence of lower respiratory symptoms was seen with SO_2until concentrations exceeded 22 ppb.

SO_2 was not significantly associated with incidence of upper respiratory symptoms.

In multipollutant models, the models with SO_2 and NO_2 had poor goodness-of-fit statistics. The log odds ratio for SO_2 was reduced by almost half in the model with PM_{10}, suggesting its association was substantially due to confounding with particles. The NO_2 log odds ratio was reduced by about one-third suggesting some confounding with PM_{10}. No increase in the incidence of lower respiratory symptoms was seen with SO_2 until concentrations exceeded 22ppb. The relationship between sulfur dioxide and incidence of lower respiratory symptoms appeared to derive only from a few influential observations, and could be confounded by PM_{10}. Aerosol acidity was not associated with the incidence of any respiratory symptoms.

Author's Conclusions:

"Exposure to particulates and SO_2 has been linked to increased bronchial secretion during colds. The consistency of these findings across the full range of acute respiratory responses argues strongly for the causality of these associations. Although confounding cannot be eliminated as an explanation for these epidemiological findings, in such time series studies the principal confounder is likely to be weather factors that have not been perfectly controlled."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **fixed monitors**Was the exposure concentration measured? (+) **study period 1 year** Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(no controls)** Were appropriate controls recruited?

	(n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (-) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (+) but not severity Was the nature and severity of symptoms graded against an explicitly defined scale? (no info) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (no) re all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (+) Are the methods reproducible? (some, more detail needed) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (no relevant conclusions re SO_2) Do the conclusions make biological sense? (no extrapolations re SO_2) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study suggests that episodes of particulate air pollution, measured by PM_{10}, $PM_{2.5}$, nephelometry, or sulfate concentrations, are associated with increased incidence of cough and lower and upper respiratory symptoms. Increased symptom reporting was observed in association with summer haze events. An association with lower respiratory symptoms was not significant until SO_2 levels were above 22 ppb and cough incidence was not statistically significant with SO_2.

However, the relationship between sulfur dioxide and incidence of lower respiratory symptoms appeared to derive only from a few influential observations and needs to be interpreted with caution.

This study attempted to identify the specific component of the pollutant mix that is responsible for acute health effects. The authors note that the validity of the study findings may have been lessened by the use of a central station to monitor pollutants as an estimate of individual exposure. Nevertheless, spatial scales were not large.

Confidence index:

Excellent detail re. the completion of the diaries.

Central monitoring stations used. Authors ensured that study areas encompassed less than $10km^2$. Subjects were chosen randomly.

Daily reports were filled out by parents. Misclassification of the reports of respiratory symptoms in children by the parents is also possible.

There was good detail on the symptoms recorded in the diaries.

The analytic methodology was described in detail.

Most confounders were considered, but more information is needed on how they were controlled for in the models.

Some of the ORs given in the body of the results section don't match with the OR in the table.

Not all SO_2 analysis referred to is included in the paper.

The authors state that the SO_2 associations were due to a few influential observations. No further detail is provided.

This study limited possible confounding factors by limiting the study to children between grade two and five. The sample was randomly selected from six US cities. While diary studies may introduce personal bias into a study, the authors provided symptoms descriptions to the participants. Groups of symptoms were then associated with respiratory conditions.

A limitation in this study may be exposure assessment—in particular, the estimate of individual exposure. Results point to particulate matter as the biggest cause for respiratory symptoms and not sulfur dioxide. However, the study was done only between April and August and not over the whole year.

Review – Confidence index ranking:

Moderate to low

NON-CLINICAL REVIEW FORM

Author: **Kienast, K., Riechelmann, H., Knorst, M., Haffner, B., Muller-Quernheim, J., Schellenberg, J., Ferlinz, R**

Internal ID: 427

Title: **Combined Exposures of Human Ciliated Cells to Different Concentrations of Sulfur Dioxide and Nitrogen Dioxide**

Year: **1996**

Abstract: In the present study we investigated the influence of two common air pollutants, sulfur dioxide (SO_2) and nitrogen dioxide (NO_2) on ciliary beat frequency (CBF). Ciliated cells were obtained by nose brush from 12 healthy volunteers and placed on a polycarbonate membrane which was in contact with Ringer's electrolyte solution. This allowed the supply of the cells by capillarity in parallel to the reaction of the pollutants with the cell surfaces. In an exposure chamber the cells were exposed for 30 min. at 37 degrees C either to SO_2 (2.5-12.5 ppm) or to NO_2 (3.0-15.0 ppm), or to a mixture of NO_2 (12.0 ppm) and SO_2 (2.5 or 5.0 ppm). CBF was measured by video-interference-microscopy. With SO2 we observed a dose-dependent decrease in CBF with Ringer's solution. 2.5 ppm SO_2 caused a 42.8% decrease and 12.5 ppm a decline of approximately 100% (8.10 +/- 0.24 Hz vs. 0.28 +/- 0.20 Hz). In parallel, we observed a decrease in the pH-value from 7.4 to 3.6. 30 min. NO_2 exposure (3.0-15.0 ppm) induced a significant dose dependent increase in CBF from 8.4 +/- 0.34 Hz to 9.4 +/- 0.44 Hz. Exposure to a mixture of SO_2 and NO_2 with Ringer's solution revealed that SO_2 exerts a stronger influence on CBF than NO_2. Exposure to both pollutants resulted in the same as exposure to SO_2 alone. Our findings demonstrate a strong correlation between SO_2-modified pH values and CBF. Exposure to a combination of two pollutants revealed the dominant influence of SO_2 on CBF while the augmented effect of exposure to NO_2 alone might be due to the oxidative potential of this gas.

Objective: This study was designed to investigate the effects of SO_2 and NO_2 exposure on human CBF following exposure to either SO_2 or NO_2 alone or to a mixture of both gases.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
0.0, 2.5, 5.0, 7.5, 10.0, 12.5 ppm	Single exposure, 30 min	Human		32±5	7 male 5 female	12	Healthy		

Observations:

Supplied by RPMI medium, a decrease in CBF of 15.2% was recorded following exposure to 12.5ppm SO_2 ($p<0.001$). Using Ringer's solution as culture medium we observed a loss in CBF up to 96.5% with 12.5ppm SO_2 ($p<0.0005$). Due to its buffer capacity, the pH value of RPMI medium remained constant at 7.5 after exposure to all SO_2 concentrations investigated. A decrease in the pH of Ringer's solution was recorded after SO2 exposure.

CBF increased in parallel to the pH value during reactivation after SO_2 exposure. Complete reactivation was not obtained after exposure to higher SO_2 concentrations; with 12.5ppm SO_2 only 43% of the reference activity was restored.

With SO_2 we observed a dose-dependent decrease in CBF with Ringer's solution.

The reaction pattern of CBF after exposure to SO_2/NO_2 gas mixture was identical to the pattern induced by SO_2-exposure alone.

Author's Conclusions:

"Our data suggest, that the decrease in CBF after SO_2 exposure was induced by an increase in hydrogen-ion concentrations in the culture medium…the present study demonstrates that the well soluble SO_2 eliminates CBF in vitro as a result of increased acidification of the tissue culture. Exposure to a SO_2/NO_2 gas combination revealed the dominant influence of SO_2 on CBF."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	**(more info needed)** Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+) b) Age *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex **(no info)** d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* **(no info)** e) Weight variation *Guideline: Below 20% variation* **(no info)** f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (+) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(no info)** Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary

	objective? **(+)** Was the duration of exposure precisely defined? **(n/a)** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(+)** Was the mode of administration appropriate? **(+)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(+) yes for temp. but no info. On SO_2** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(n/a)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(n/a)** Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* **(n/a)** Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* **(n/a)** Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* **(n/a)** Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(+)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(-)** Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?

E. Procedural:	**(n/a)** Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* **(n/a)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(n/a)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(n/a)** Was a control group assayed? (+) Is the control regime appropriate? **(n/a)** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(n/a)** Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible?)+) Did either the study design or the statistical analysis limit confounding

	variables? . **(more info needed)** Do the conclusions make biological sense? . **(no extraps made)** Are the extrapolations reasonable? . **(+)** Is the paper in a peer-reviewed journal? . **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings showed a dose dependent decrease in ciliary beat frequency and exposure to SO_2 (in Ringer's solution) . There was also a strong correlation between SO_2 modified pH values and CBF. These results provide evidence to the mechanism of SO_2 exposure in *in vitro* studies; however, the results do not necessarily apply to *in vivo* studies.

This study attempted to increase the available literature on effects of NO_2/SO_2 mixtures, as well as exposure to SO_2 alone. This study was significant in that urban areas and workplaces frequently contain these gases. However, the low number of subjects used reduces the validity and generalizability of the results. Furthermore, the study did not indicate if nasal morphology or health had been accounted for.

Exposure to an SO_2/NO_2 gas mixture showed a decrease in CBF similar to that observed with SO_2 alone. Study finds a strong correlation between SO_2-modified pH values and CBF.

Confidence index:

There is no information on whether the SO_2 was continuously monitored and maintained during the exposure.

The exposure chamber allowed the cells to be directly exposed to the gas.

Preliminary studies were conducted to determine suitable doses.

Consistency was reported with some other studies.

This study attempted to limit confounding factors of the actual cilial cells by closely monitoring environmental conditions of the cell culture in order to maintain normal metabolic and functional cell activities. However, the origins of the nasal cell were not well documented. Statistical methods used produced evidence of strong correlation between decrease in CBF and in pH-values of tissue culture under SO_2 exposure. Results are not entirely novel but support previous studies that demonstrated a correlation between SO_2 exposure and decreased ciliary motility.

As this was an in vitro study, a lot of the criteria such as group assignment were not evaluated. No evidence was provided in the report to justify a dosing regime, and there was no report on preliminary trials to identify the range of exposure levels. Airflow was not monitored continuously in the exposure chamber. Finally, nominal and actual concentration values were not recorded.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Pinter, A., Rudnai, P., Sarkany, E., Goczan, M., Paldy, A.	**Internal ID:** 428
Title: Air Pollution and Children's Respiratory Morbidity in the Tata Area, Hungary	
Year: 1996	
Abstract: Air pollution represents one of the most important environmental health hazards in Hungary. Irritant gases, like SO_2 and NO_2 levels exceed national and international standards in many settlements. Tata, a small town, situated in a basin, is one of the most polluted areas in Hungary. Longitudinal and cross-sectional studies have been conducted in children in the winter period of 1993/1994, with respect to SO_2 and NO_2 concentration. Average SO_2 levels exceeded the national standard levels and daily peaks as high as 450 $\mu g/m^3$ were recorded. Excessive NO_2 levels were also found but they were not as high as those of SO_2. Acute respiratory morbidity, based on a uniform protocol was recorded daily and evaluated on a daily and weekly basis. A statistically significant correlation with SO_2 levels was observed in relation to the frequency of acute daily respiratory morbidity. Other health parameters, like pulmonary function, haematology and sensory performance were also tested. Although no statistically significant correlations were observed, the tendency in all parameters demonstrated impairment, in relation with ambient air pollution. Smoking history of the family did not alter significantly the pulmonary functions of other parameters.	
Objective: The aim of this study was:- to study relationship between irritant gases and respiratory morbidity in children; - to utilize general health indicators for establishing relationships between air pollution and health effects in children.	

Overall study design:

Exposure level	Exposure frequency/ duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
1st. Study Longitudinal Prospective Study Not clearly reported. However, mean values of SO_2 concentrations exceeded considerably the limit values of the yearly average 150 $\mu g/m^3$, max values were generally twice as high as that indicating the importance of episodic high hazard. **2nd. Study Cross-Sectional Study** 9-11y	Study period: Dec 1993-March 1994	No info	<14 yrs				

Observations:

Longitudinal Prospective Study

Peak concentrations of SO_2 were observed in weeks 45-50 (maximum average up to approx. 175µg/m^3), which did not correlate with weekly registered respiratory morbidity.

When correlation was made with real-time monitoring, the correlation between SO_2 concentrations and daily respiratory morbidity turned out to be significant during the first 13 weeks in 1994. If the weighted mean concentrations of SO_2 of previous 3 or 7 days were analyzed, the correlation was more pronounced.
The smoking history of the family might influence the lung function of the children. Comparison was made between those children, who are living in a smoking family and high SO_2-exposed area. Children from schools exposed to higher SO_2 levels, had impaired values, however, the smoking history seemed to have no significance. Other health parameters, like pulmonary function, haematology and sensory performance, were also tested. Although no statistically significant correlations were observed, the tendency in all parameters demonstrated impairment, in relation with ambient air pollution. Smoking history of the family did not alter significantly the pulmonary functions of other parameters.

Cross Sectional Study

There are no relevant results or observations to report. See below in Discussion of findings and Confidence Index for further information.

*Statistically significant

Author's Conclusions:

"In the present study, significant correlation was observed between daily concentration of SO_2 and the incidence of acute respiratory morbidity in children. Daily number of cases and weighted mean SO_2 levels of previous 3 days showed less convincing correlation. No significant correlation was observed when weekly incidence was analysed. Ambient NO_2 concentration showed no correlation at all with respiratory morbidity. The role of other confounding factors cannot be ruled out. Ambient temperature influences the development of respiratory morbidity in a complex way; concentrations of pollutants due to intensity of domestic heating, frequency of infections, etc."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitors Was the exposure concentration measured? (+) study period. 4 months Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?

D. Procedural:	(no info) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (-) Was the timing and length of follow-up appropriate? (more info needed) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (more info needed) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (more info needed) Were accepted assessment methods employed? (more info needed) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (more info needed) Did qualified personnel conduct the exams? (more info needed) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (-) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-) Are the statistical methods used appropriate? (-) Are confidence intervals reported? ((more info needed) Did the investigator appropriately interpret the statistics? (more info needed) Are the observed trends statistically significant?
G. Interpretations:	(-) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (more info needed) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (-) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The study was very poorly designed and extremely confusing.

Study findings included significant correlations between daily concentrations of SO_2 and incidence of acute respiratory morbidity. While NO_2 did not appear to confound findings, the role of other confounding factors, such as ambient temperature, concentration of pollutants of domestic heating and frequency of infection should be analyzed in future studies.

Dysfunction of haematopoiesis was consistently found in the whole child population and this finding deserves further attention in the health protection of children.

Confidence index:

There is extremely limited detail in all sections of the paper.

It is not obvious until the results section that there were two studies, a longitudinal prospective and a cross sectional.

SO_2 levels are not clearly presented.

The monitoring systems consisted of 3 discontinuous measuring stations and one real-time monitor. The real-time data was only available for the first 13 weeks in 1994 and these data are all that is presented.

The homes are heated by low-quality coal.

The diagrams are confusing and not well described.

There is no detail given on the study populations. There is no information on how the study population for the cross sectional population were chosen or where they are from, or how many there were.

All that is mentioned in the cross sectional study re. SO_2 is that mean cell volume of erythrocytes were studied in connection with SO_2 exposure and that there was no difference between high and low pollution among girls but a considerable difference in boys. There are no other details provided, i.e. methods, study population, etc.

The literature review is extremely inadequate.

Diagrams are included in the paper, but not referred to or described in the paper.

This study utilized data from five different pediatricians, introducing personal bias into the study. Real-time monitoring was used only from week 49 onwards. Data collected prior to this point using discontinuous monitoring stations did not correlated with weekly registered respiratory morbidity.

Timing and frequency of data collection were not adequate. Confidence intervals were not reported, and information on measuring devices was very vague. There are limitations in the exposure assessment.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Park, H., Lee, B., Ha, E.H., Lee, J.T., Kim, H., Hong, Y.C. | **Internal ID:** 429

Title: Association of Air Pollution with School Absenteeism due to Illness

Year: 2002

Abstract: OBJECTIVE: To assess the association of air pollution and school absenteeism among elementary students. DESIGN: Time-series analysis of air pollution and school absenteeism data with controlling for long-term trends, seasonality, day of the week, and holiday as well as meteorologic variables. SETTING: School absenteeism data for the period from March 2, 1996, to December 22, 1999, were collected from student attendance reports of one elementary school in Seoul, Korea. MAIN OUTCOME MEASURES: The number of daily illness-related absences was analyzed against the daily levels of air pollution by generalized additive Poisson regression. The relative risks of absenteeism for air pollution exposure of interquartile ranges (the range from the lowest 25% of the value to the lowest 75% of the value) on absenteeism were calculated on the same day. RESULTS: Exposure to air pollutants such as particulate matter of 10 micro m or less in aerodynamic diameter (PM_{10}), sulfur dioxide, and ozone, was associated with illness-related absenteeism. The estimated relative risks were 1.06 (95% confidence interval, 1.04-1.09) per 42.1- $\mu g/m^3$ increase in PM_{10}, 1.09 (95% confidence interval, 1.07-1.12) per 5.68-part per billion increase in sulfur dioxide, and 1.08 (95% confidence interval, 1.06-1.11) per 15.94-part per billion increase in ozone. There was no significant relationship between nitrogen dioxide level and illness-related absenteeism. CONCLUSION: Air pollution is associated with illness-related absences among elementary students.

Objective: To assess the association of air pollution and school absenteeism among elementary students. DESIGN: Time-series analysis of air pollution and school absenteeism data with controlling for long-term trends, seasonality, day of the week, and holiday as well as meteorologic variables.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Mean: 9.19±4.61ppb Range: 2.68-28.11ppb	Study period: March 2, 1996 – December 22, 1999	671 boys 593 girls	Grades 1-6	Average enrollment count at school: 1264	No info	n/a	n/a

Observations:

Average number of daily absences due to illness were 4.20±3.50. On the basis of enrollment in 1996, the absence rate was 4.66 per 10000 student-days and the illness-related absence rate was 3.69 per 100 student-days.

The 24h average concentrations of PM_{10}, NO_2, SO_2, and CO were 68.11 ug/m^3, 33.12ppb, 9.19ppb, and 1.11ppm, respectively. The concentrations of CO, NO_2, SO_2, and PM_{10} positively correlated with each other. However, the concentration of ozone negatively correlated with that of the other pollutants. Relative risk of the absence for the interquartile range increase of SO_2 was 1.09%. For non-illness related absenteeism, air pollutants were not associated statistically significantly with absences, except for ozone and sulfur dioxide. Concentrations of ozone and SO_2 were negatively associated with non-illness related absences.

Illness related absences occurred more frequently when levels of sulfur dioxide increased. Relative risks of the absences for the interquartile range increase (5.68 ppb) of SO_2 was 1.09 (95%CI 1.07-1.12). Other estimated relative risks were 1.06 per 42.1μg/m^3 increase in PM_{10}, and 1.08 per 15.94 part per billion increase in ozone.

There was a relatively linear positive relationship between the number of daily illness-related absences and concentrations of sulfur dioxide.

Exposure to air pollutants such as particulate matter of 10μm or less in aerodynamic diameter, sulfur dioxide, and ozone, was associated with illness-related absenteeism.

*Statistically significant

Author's Conclusions:

"In conclusion, air pollutants were associated with illness-related absences, which suggests that air pollution is closely linked to daily activities, particularly of school-aged children."

"Our results show that air pollutants such as ozone and SO_2 are protective for the non-illness related absences. Other pollutants also tended to decrease the number of non-illness-related absences. Because the causes of non-illness-related absences were not ascertained in our study, it is hard to explain why air pollutants decrease the number of such absences. One possible explanation is that air pollution decreases outdoor activities such as family tours or visits, thereby increasing attendance a school. Further studies including detailed causes of absences are needed."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (more info needed) Were identical 'criteria for exclusion' applied to both cases and controls? (-) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitors Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (+) absences not related to illness Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (more info needed) Was the timing and length of follow-up appropriate? (more info needed) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)

E. Data collection:	(more info needed) Was the assessment identical for cases and controls? (more info needed) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (-) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (n/a no exams) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? (-) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Exposure to SO_2 was related to illness-related absences among elementary students. However, there was no detail on the cause of absences. The results also show that SO_2 was protective for the non-illness related absences; however, further study is needed and further information is needed on the exact reason for absence.

While previous studies have focused on the effects of air pollution on school absenteeism, this study controlled for long-term trends, seasonality, day of the week, holiday and meteorologic influences. One of the limitations of this study was that authors failed to record specific reasons for non-illness related absences. Furthermore, individual-level covariates were not considered.

A few studies have shown that air pollution is related to school absence, but they have produced inconsistent results. Illness related school absences are considered a comprehensive index for evaluating health-related effects of air pollution on children. This study suggests that air pollution increases illness-related school absences.

Confidence index:

Only one school was chosen for the study. This school was chosen because the traffic is particularly heavy in this area.

The illness related absence was recorded based on a parent's report.

Comprehensive monitoring system – 27 sites. However, there is no other detail given.

The study states it controls for long-term trends, seasonality, day of the week, holiday and meteorologic influences, but does not provide characteristics of the subjects such as sociodemographic data. There is no detail on how confounders were controlled.

The relative risks are low.

The number of studies showing the relationship between school absenteeism and air pollutants was limited and therefore there were few studies available for comparison.

The specific reasons for non-illness related absences could by be exposure because of the dependence on past records.

There was no detailed information about the reason for absences in the attendance report.

Teachers distinguish between an absence related to illness and that not related to illness by relying on the parent's report.

This study mentioned that a protocol was approved by an institutional board, but provides no information on the protocol itself.

The authors point out some limitations in this report. Specific reasons for non-illness-related absences could not be explored because of limited access to data. In addition, distinguishing between an absence related to illness and that not related to illness was up to the teacher's discretion. There are weaknesses in this study on data collection. As well, raw data and individual data were not given.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Zeghnoun, A., Czernichow, P., Beaudeau, P., Hautemaniere, A., Froment, L., Le Tertre, A., Quenel, P.	**Internal ID:** 430
Title: Short-Term Effects of Air Pollution on Mortality in the Cities of Rouen and le Havre, France, 1990-1995	
Year: 2001	
Abstract: In this study, the authors examined the short-term effects of ambient air pollution on mortality across 2 French cities: Rouen and Le Havre. In Poisson regression models, which controlled for day-of-week effects, the authors used nonparametric smoothing to control for temporal trend, weather, and influenza epidemics. In Rouen, an interquartile range increase of 60.5-94.1 $\mu g/m^3$ of ozone was associated with an increase of 4.1% (95% confidence interval = 0.6, 7.8) of total mortality. Daily variations in sulfur dioxide (interquartile range increase = 17.6-36.4 $\mu g/m^3$) were also associated with an 8.2% increase (95% confidence interval = 0.4, 16.6) in respiratory mortality. An increase of 6.1% (95% confidence interval = 1.5, 10.9) of cardiovascular mortality was also observed with an interquartile range increase of nitrogen dioxide (i.e., 25.3-42.2 $\mu g/m^3$). With respect to Le Havre, an interquartile range increase in daily levels of sulfur dioxide (11.3-35.6 $\mu g/m^3$) was associated with an increase of approximately 3% (95% confidence interval = 0.8, 5) of cardiovascular mortality. For particulate matter less than or equal to 13 µm in diameter (interquartile increase = 21.5, 45.4 $\mu g/m^3$), an increase of 6.2% (95% confidence interval = 0.1, 12.8) was observed. The estimates of pollutant effects and their standard deviations were slightly affected by the degree of smoothing temporal variations in this study. When low collinearity was present, the 2-pollutant models provided acceptable estimates of pollutant effects. They suggested that the ozone effect was independent of the Black Smoke effect, and that the effects of sulfur dioxide and nitrogen dioxide were unlikely to be confounded by ozone concentrations. However, high collinearity leads to large estimates of the pollutant coefficient variances and, therefore, leads to inaccurate estimates of pollutant effects. The analysis of the contributory effects of different pollutant mixtures requires further investigation in those instances in which high collinearity between pollutants is present.	
Objective: In the current study , we sought to evaluate the short-term associations between acid-particulate and photooxidant air pollution and daily mortality in the cities of Rouen and Le Havre.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Rouen **Summer-** Mean-23.83$\mu g/m^3$	Study period: 1990-1995	No info	Approx. 70% of deaths in each city were	Number of deaths during study period: Rouen-21,853	No info	n/a	n/a

Winter- Mean-35.36µg/m^3 Le Havre **Summer-** Mean-27.83µg/m^3 **Winter-** Mean-39.66µg/m^3			in the at least 65y age group	Le Havre-13885 deaths			

Observations:

Rouen: Sulfur dioxide, with a 0-2 day lag (IQR increase = 17.6-36.4 µg/m^3), was associated with an increase of 8.2% (95% CI = 0.4-16.6) in respiratory mortality. An interquartile range increase of 60.5-94.1 µg/m^3 of ozone was associated with an increase of 4.1% of total mortality.

Le Havre-an IQR increase in daily levels of SO_2 (11.3-35.6 µg/m^3) was associated with an approximate increase of 3% (95% CI=0.8-5) in cardiovascular mortality.
For particulate matter less than or equal to 10µm in diameter (interquartile increase = 21.5, 45.4 µg/m^3), an increase of 6.2% was observed. The estimates of pollutant effects and their standard deviations were slightly affected by the degree of smoothing temporal variations in this study. When low collinearity was present, the 2-pollutant models provided acceptable estimates of pollutant effects.

Author's Conclusions:

"In conclusion, analysis of mortality among the populations of Rouen and Le Havre from 1990 to 1995 revealed an increase in deaths relative to air pollutant levels. In Rouen, the strongest association occurred between SO_2 and respiratory mortality, followed by NO_2 and cardiovascular mortality, and subsequently O_3 and total mortality. In Le Havre, the associations observed between both SO_2 and PM_{13} and cardiovascular mortality were the most significant. These associations were robust in the sense that they were minimally affected by the degree of smoothing that we used to control for temporal trend and for seasonal variations, as well as by addition of autoregressive terms in the models."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made?

	(n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? **(+)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	**(+) fixed monitors**Was the exposure concentration measured? **(+) study period 1990-1995**Is the exposure duration known?
C. Equipment:	**(-)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(+)** Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) **(+)** Was the timing and length of follow-up appropriate? **(+)** Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? **(+) mortality counts** Were appropriate endpoints examined? **(+)** Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(+)** Were accepted assessment methods employed? **(+)** Were assessment measures sufficiently sensitive? **(n/a mortality data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(+)** Did qualified personnel conduct the exams? **(+)** Was the timing and frequency of assessment logical? **(-)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	**(+)** Are the statistical methods used appropriate? **(+)** Are confidence intervals reported? **(+)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	**(+)** Was the original objective addressed? **(-)** Are the results novel? **(+)** Was the design of the study appropriate to investigate the primary objective? **(+)** Were assumptions reasonable?

	(-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Excess mortality was associated with SO_2. Positive and significant associations were observed between $SO_2$and respiratory mortality in Rouen, and between $SO_2$and cardiovascular mortality in Le Havre. Only a small number of deaths were observed daily, particularly with respiratory mortality, which was associated with relatively large confidence intervals. Population subgroups that may be more susceptible to air pollution such as the elderly were also not studied.

Several earlier studies have produced similar associations between cardiovascular mortality and particulates. The analysis of the contributory effects of different pollutant mixtures requires further investigation if high collinearity between pollutants is present.

Confidence index:

Though a pollution network of monitors was available, in the end, only one SO_2 monitor per city was used for this study. The control of confounders was explained in clear detail.

Good description and comparison of study areas. Statistical methods were suitable and explained clearly .

This study used a very large sample size. Consequently, it was not feasible to collect characteristics such as mean age and socioeconomic data. However, the author described the industries in the communities and took into consideration meteorological differences between the two cities.

This discussion in this study would have been very thorough had it also discussed limitations of the study. No raw or individual data was reported, and the sensitivity of the monitoring devices was not stated.

Review – Confidence index ranking:

Moderate

CASE-CONTROL REVIEW FORM

Author: Tenias, J.M., Ballester, F., Perez-Hoyos, S., Rivera, M.L. | **Internal ID:** 431

Title: Air Pollution and Hospital Emergency Room Admissions for Chronic Obstructive Pulmonary Disease in Valencia, Spain

Year: 2002

Abstract: The short-term relationship between levels of air pollution and emergency room admissions for chronic obstructive pulmonary disease was assessed in Valencia, Spain. The design was an ecological time-series study in which daily variation in air pollution was related to emergency chronic obstructive pulmonary disease visits to one of the city's hospitals. The pollutants under investigation were Black Smoke, sulfur dioxide, nitrogen dioxide, carbon monoxide, and ozone. The degree of association was analyzed with Poisson autoregressive regression, for which trend, seasonal patterns, temperature, humidity, days of the week, and incidence of influenza were controlled. Increases of 10 $\mu g/m^3$ in ozone levels (lag 5) and of 1 mg/m^3 in carbon monoxide (lag 1) were associated with increases of 6.1% (95% confidence interval [CI] = 2.2%, 10.1%) and of 3.9% (95% CI = 1.4%, 6.6%), respectively, in the expected chronic obstructive pulmonary disease cases. There was no significant association for the remainder of the pollutants. The described effects persisted even when the authors used models of differing specifications and when generalized additive models were used. The authors concluded that the results of this investigation, together with results of earlier research, demonstrate the significant effect of pollution on various health indicators within Valencia.

Objective: In the current investigation we sought to 1. calculate the short-term effects of air pollution on emergency room visits for COPD within Valencia, Spain, and 2. analyze other aspects that are relevant to this relationship.

Overall study design:

Exposure level	**Exposure frequency /duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
24hr mean: 26.6$\mu g/m^3$ 24hr range:5.2-68.4$\mu g/m^3$ 1hr mean: 56.3$\mu g/m^3$ 1hr range:8.8-157.4$\mu g/m^3$	Study period: 1994-1995	No info	>14y	1298 COPD admissions	No info	n/a	n/a

Observations:

The relative risks for an increase in SO_2 of 10$\mu g/m^3$ was less than 1 and not significant.

Increases of 10 $\mu g/m^3$ in ozone levels (lag 5) and of 1 mg/m^3 (lag 1) in carbon monoxide were associated with increases of 6.1% and of 3.9%, respectively, in the expected chronic obstructive pulmonary disease cases.

There was no significant association for the remainder of the pollutants. The described effects persisted even when the authors used models of differing specification and when generalized additive models were used.

Association was observed between black smoke, SO_2, and CO, and a negative correlation was noted with O_3. Temperature produced a positive correlation with O_3 and a negative association with CO, Black smoke, and SO_2.

Author's Conclusions:

There were no specific conclusions re. SO_2 exposure.

"In conclusion, the results of this investigation, together with results of earlier research, demonstrate the significant impact of pollution on various health indicators within Valencia. Moreover, the linear dose-response found hinders the establishment of a safety threshold below which it can be assumed that there is no adverse effect on the health of the population. We, therefore consider that a reduction in levels of atmospheric pollution would have a positive effect on public health."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(more info needed)** Is the number of subjects sufficient to produce statistically significant results? **(-)** Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(more info needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? **(+)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	**(+) fixed monitors** Was the exposure concentration measured? **(+) study period 2 years** Is the exposure duration known?
C. Equipment:	**(-)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(+)** Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no binding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(+)** Was the timing and length of follow-up appropriate? **(+)** Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? **(+)** Were appropriate endpoints examined? **(-)** Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(+)** Were accepted assessment methods employed? **(+)** Were assessment measures sufficiently sensitive? **(more info needed)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(+)** Did qualified personnel conduct the exams? **(+)** Was the timing and frequency of assessment logical? **(-)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?

F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (more info needed) Were assumptions reasonable? (+) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (no specific conclusions re SO_2) Do the conclusions make biological sense? (No extrapolations for SO_2) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

SO_2 was not significantly associated with ER visits for COPD. Previous studies have examined the relationship with emergency room visits for asthma rather than for chronic obstructive pulmonary disease. The author evaluated the quality of health indicator data and found it to have good reliability. Furthermore, there was no ambiguity in determining the day on which the visit to hospital took place, which could have compromised the validity of the data.

The authors concluded that the results of this investigation, together with results of earlier research, demonstrate the significant effect of pollution on various health indicators within Valencia. The most consistent findings were those related to O_3 and CO; these findings showed a positive and significant effect over the entire period.

Confidence index:

Standard methodology used – Air Pollution and Health – a European Approach (APHEA) project.

Only data from one hospital was studied with an urban catchment area of approximately 200,000 inhabitants. The total population of Valencia was 750,000. The study subjects aren't necessarily representative of the general population.

Previously validated data collection methods were used and the reliability of the method had been tested.

The monitoring systems consisted of 6 manual and 5 automatic monitoring stations. More information is needed on the correlations between the measurements at different stations. The authors considered possible confounding factors such as local holidays, strike action and meteorological data. Meteorological and sociocultural factors were accounted for using Poisson autoregressive analysis. Confidence intervals were included and significant findings were produced for some of the pollutants. This study found no significant association between COPD and SO_2. Comments in the discussion focused mainly on ozone and carbon monoxide. Data sources and collection were well-detailed as were statistical methods. There are no raw or individual data, and sensitivity of monitoring devices is not mentioned.

Review – Confidence index ranking:

Moderate to low

CASE-CONTROL REVIEW FORM

Author: Mortimer, K.M., Neas, L.M., Dockery, D.W., Redline, S., and Tager, I.B.	**Internal ID:** 432

Title: The effect of air pollution on inner-city children with asthma.

Year: 2001

Abstract: The effect of daily ambient air pollution was examined within a cohort of 846 asthmatic children residing in eight urban areas of the USA, using data from the National Cooperative Inner-City Asthma Study. Mixed linear models and generalized estimating equation models were used to evaluate the effects of several air pollutants—ozone, SO_2, NO_2, and PM_{10}—on peak expiratory flow rate and symptoms in 846 children with a history of asthma. Results support previous time-series findings that at levels below current USA air-quality standards, summer air pollution is significantly related to symptoms and decreased pulmonary function among children with asthma.

Objective: It was found that asthmatic children born prematurely or with a low birth weight had a significantly greater response to increases in ozone. This study compares these results to time-series and other analyses and presents multipollutant models.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
~ 5 – 75 ppb (range between cities) 53 ppb (average)	daily/2 months	male and female	4-9 years	846	asthmatic	-	-

Observations:

Daily SO_2 data were available for all eight urban areas, with an average intradiary range of 53ppb. The correlation between 8h ozone and 3h mean SO_2 was 0.29. Single-pollutant models suggest that single and multiday lags of SO_2 had little effect on morning %PEFR. The greatest effect on morning symptoms was seen for a 2-day moving average lag: increase in 2-day average SO_2, OR=1.48 per IQR increase in 6-day average NO_2 and OR=1.26 per IQR increase in 2-day average PM_{10}. Results were similar for 3 and 4-day moving averages. When 5-day average ozone and 2-day average SO_2 were entered in a model simultaneously, there was essentially no impact on the estimate for SO_2 while the estimate for ozone decreased slightly.

None of the pollutants were associated with evening PEFR or symptom reports. Only ozone was associated with declines in morning % PEFR per interquartile range increase in 5-day. In single pollutant models, each pollutant was associated with an increased incidence of morning symptoms: for SO_2 per interquartile range increase in 2-day average, odds ration = 1.48.

*Statistically significant

Author's Conclusions:

"This longitudinal analysis supports previous time-series findings that at levels below current USA air-quality standards, summer air pollution is significantly related to symptoms and decreased pulmonary function among children with asthma."

"In conclusion, summer-time air pollution is associated with increased asthma morbidity and decreased pulmonary function among inner-city children with asthma in the USA. These findings from generalized estimating equations and mixed models support previously published reports from time-series analysis and those reported from less urban populations. The impact of pollution was not immediate, but developed over several days, with the largest effects seen on morning outcomes. Nitrogen dioxide, sulphur dioxide, and particles with a 50% cut-off aerodynamic diameter of $10\mu m$ were associated with increases in symptoms, with nitrogen dioxide exhibiting the strongest influence. Ozone was most influential on peak expiratory flow rate. Adverse respiratory effects were observed in all cities, at levels below proposed USA air quality standards."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a)Were identical 'criteria for exclusion' applied to both cases and controls? (n/a)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (+)Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(n/a)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a)Were appropriate controls recruited? (n/a)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (n/a)Was the timing and length of follow-up appropriate? (n/a)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (+/-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (-)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+/-)Was the timing and frequency of assessment logical?

	(-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (n/a)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (+)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (+/-)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? (+)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (+)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study provides evidence of measurable negative respiratory health effects in children from eight inner-city communities in northeastern and Midwestern USA. While ozone was most influential on PEFR, NO_2 had the strongest effect on symptoms.

This study determined that summer-time air pollution was associated with increased asthma morbidity and decreased pulmonary function among inner-city children with asthma in the USA.

Confidence index:

Exposure assessment may definitely be a weakness here with parents of children between the ages of 4 and 9 keeping diaries. Classification and assessment measures may not be sufficiently sensitive. Also, there may be confounding factors such as exposure to cigarette smoke.

This study utilized a large sample set from eight urban US cities. To limit possible confounding factors, the authors limited the participants to children between the ages of 4-9 who resided in inner-city neighborhoods with incomes of at least 30% of the residents was below the federal poverty level. The authors also reduced confounding by temperature and seasonal infectious disease by restricting the analysis to children who returned at least one diary during June-August 1993.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Tarlo, S.M., Broder, I., Corey, P., Chan-Yeung, M., Ferguson, A., Becker, A., Rogers, C., Okada, M., Manfreda, J.	**Internal ID:** 433

Title: The role of symptomatic colds in asthma exacerbations: Influence of outdoor allergens and air pollutants

Year: 2001

Abstract: BACKGROUND: Both air pollutants and respiratory viral infections have been recognized to be important triggers of asthma exacerbations, but the possible interaction of these has not been assessed in an epidemiologic study. OBJECTIVE: In this study, we aimed to assess the possible associations of symptomatic colds with outdoor allergens and air pollutants in exacerbating asthma. METHODS: A total of 57 adults and children with asthma participated in a 1-year study in Vancouver, British Columbia. Diary cards recording symptoms of asthma and colds, medication usage, and peak expiratory flow rates were completed twice daily. Outdoor air pollutant levels and levels of pollen and fungal spores were measured. Levels were examined for associations with exacerbations of asthma with symptoms of a cold, as compared with asthma exacerbations with no cold symptoms and colds in the absence of asthma exacerbations. RESULTS: Of 130 asthma exacerbations, 47% were associated with cold symptoms (accounting for 44% of episodes of colds). Asthma exacerbations with colds were associated with higher levels of sulfur dioxide, and nitric oxide during March to November in comparison with asthma exacerbations without cold symptoms ($P < .018$). Cold-symptom days overall were more common in winter and were significantly associated with lower temperatures, lower ozone levels, and higher sulfur dioxide, nitrogen dioxide, nitrogen oxide (NO and NO_x), and carbon monoxide levels. CONCLUSIONS: Asthma exacerbations are commonly associated with cold symptoms. These episodes are associated with higher levels of sulfur dioxide and nitrogen oxides from March to November in comparison with asthma exacerbations without cold symptoms.

Objective: The present study was performed to assess possible associations between environmental factors such as air pollutants and symptomatic colds I the production of asthma exacerbations.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
See observations section	Sept 1991-March 1993	49% male	26.8 ± 19y (7-55 years) 35% were children	57	Asthmatic	n/a	n/a

Observations:

Days in which cold symptoms were recorded had significantly higher mean levels of SO_2 (5.3 ± 4.7 ppb vs. 4.7 ± 4.2ppb; $p=0.0002$).

The outdoor environmental pollutant levels were averaged per patient for 1, 3, and 5 days before each acute event (colds with and without asthma exacerbations and asthma exacerbations without a cold) and compared for these 3 types of acute events. There was a statistically significant association for asthma exacerbations with colds and levels of SO_2 which were significantly higher for a 3-day mean (4.94ppb) before exacerbations with cold than for a 3-day mean before exacerbations without colds (3.04 ppb) ($p<01$) during warmer months. This was not evident in the cooler months and was not evident for 1 day before the acute event.

The relation of colds with asthma exacerbations was significant for preceding SO_2 in children.

Each individual subject could have more than 1 type of acute event; 13 subjects had colds with and without asthma exacerbations, 7 had asthma exacerbations with and without colds, and 13 had asthma exacerbations without a cold and colds without asthma exacerbations. After correcting for this, asthma exacerbations with a cold continued to be statistically significantly associated with preceding 3-day mean levels of SO_2 versus asthma exacerbations without a cold.

Of 130 asthma exacerbations, 47% were associated with cold symptoms (accounting for 44% of episodes of colds). Asthma exacerbations with colds were associated with higher levels of SO_2, and nitric oxide during March to November in comparison with asthma exacerbations without cold symptoms. Cold-symptom days overall were more common in winter and were significantly associated with lower temperatures, lower ozone levels, and higher SO_2, NO_2, NO_x, and CO levels.

Author's Conclusions:

"Asthma exacerbations are commonly associated with cold symptoms. These episodes are associated with higher levels of sulfur dioxide and nitrogen oxides from March to November in comparison with asthma exacerbations without cold symptoms."

"The proportion of asthma exacerbations related to symptomatic colds was greater during the late fall, winter, and spring months. The possibility that some of these symptomatic colds truly were manifestations of allergic rhinitis has been considered, especially for episodes of increased nasal symptoms and sore throat without fever, inasmuch as PCRs or other laboratory confirmations of a viral etiology were not obtained in this study. Asthma exacerbations with colds were not associated with pollen or fungal spore levels, making it unlikely that many of these episodes resulted from seasonal allergic responses, though some episodes might have been due to seasonal allergic rhinitis and some to nonseasonal allergic responses."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(more info needed)** Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? **(more detail needed)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **fixed monitors** Was the exposure concentration measured? (+) 1 year Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval?

	(n/a no controls) Were appropriate controls recruited? (n/a no blinding indicated) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+/-) Were assessment measures sufficiently sensitive? (no info) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (no withdrawals listed) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (+) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (more detail needed) Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study showed that symptomatic colds are important triggers of asthma in children and in adults. An association from March to November was found between higher levels of SO_2 for 3 days before asthma exacerbations with a cold in comparison with the levels of SO_2 before asthma exacerbations without a cold. It is possible that the apparent association seen between symptomatic colds with asthma exacerbations and $SO_2$was due to a coincidental seasonal rise. The association may differ depending on the specific viral infection. During this 1-year study of symptoms as recorded on diary cards, symptomatic colds were associated with 46.9% of asthma exacerbations. The proportion of asthma exacerbations related to symptomatic colds was greater during the late fall, winter, and spring months.

This study attempted to assess possible associations between environmental factors and symptomatic colds in the production of asthma exacerbations. The lack of associations demonstrated by the findings might suggest an interaction of pollutants with only selected viruses in enhancing the asthmatic response. Future studies could use PCR to identify specific viral infections.

Confidence index:

Sample size may have been too small. Not tested for statistical power.

Training effect was considered and the subjects were given a 2-week training period.

Diagnosis of colds relied on subjects reported in diary.

More detail is needed on how the subjects were chosen from each clinic. Was it random?

Standardized methods was used for the detection methods and equipment used for SO_2monitoring.

Excellent detail on definitions of asthma exacerbations and symptomatic colds. These definitions had been validated in other studies.

It does not appear that the subjects were blinded to the main objective of the study.

This study characterized the subjects involved, describing pre-study health conditions, age, and where they lived. The paper describes the recruitment process and describes the pre-experimental coaching process to teach subjects how to complete the diary card, to reduce personal bias between subjects.

There are limitations in the exposure assessment. Raw and individual data are missing.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Vedal, S., Brauer M., White, R., Petkau, J. | **Internal ID:** 434

Title: Air Pollution and Daily Mortality in a City with Low Levels of Pollution

Year: 2003

Abstract: The concentration-response relationship between daily ambient inhalable particle (particulate matter less than or equal to 10 µm; PM_{10}) concentrations and daily mortality typically shows no evidence of a threshold concentration below which no relationship is observed. However, the power to assess a relationship at very low concentrations of PM_{10} has been limited in studies to date. The concentrations of PM_{10} and other air pollutants in Vancouver, British Columbia, Canada, from January 1994 through December 1996 were very low: the 50th and 90th percentiles of daily average PM_{10} concentrations were 13 and 23 $\mu g/m^3$, respectively, and 27 and 39 ppb, respectively, for 1-hr maximum ozone. Analyses of 3 years of daily pollution PM_{10}, ozone, sulfur dioxide, nitrogen dioxide, and carbon monoxide) concentrations and mortality counts showed that the dominant associations were between ozone and total mortality and respiratory and cardiovascular mortality in the summer, and between nitrogen dioxide and total mortality in the winter, although some association with PM_{10} may also have been present. We conclude that increases in low concentrations of air pollution are associated with increased daily mortality. These findings may support the notion that no threshold pollutant concentrations are present, but they also raise concern that these effects may not be effects of the measured pollutants themselves, but rather of some other factor(s) present in the air pollution-meteorology mix.

Objective: To look at the association between low levels of pollutants and daily mortality in Vancouver BC, a city with low levels of pollution. To determine the concentration-response relationship between daily ambient inhalable particle concentrations and daily mortality.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Mean: 2.8±1.7 Range: 0.3-15.4ppb	Jan 1994-Dec 1996	Male and female	No info	#s of daily deaths ranged from 30-40/day	No info	n/a	n/a

Observations:

All of the SO_2 effects were calculated for a standard deviation increase in SO_2.

In the one-pollutant model, during the summer, a statistically significant effect on total deaths in the summer was observed for PM_{10}, O_3, and SO_2. In winter, significant effects were seen on total deaths for PM_{10} (lag2), NO_2 (lag2), and SO_2 (lag1). No significant deleterious effects on respiratory deaths were observed. Effects that were significant in the single-pollutant models were substantially diminished after addition of other pollutants in the two pollutant models. The greatly diminished effect of SO_2 at lag1 on total mortality in the winter after the addition of NO_2 was observed. Effects on cardiovascular deaths were observed for SO_2 lag 1 (not significant). The percentage changes were small with wide confidence intervals in some cases.

Author's Conclusions:

"In conclusion, assuming that the findings from Vancouver are generalizable to other cities with low pollutant concentrations, increases in air pollutant concentrations, even when concentrations are low, are associated with adverse effects on daily mortality. Although this observation may support the argument that there are no threshold concentrations of air pollution below which adverse effects cannot be detected, it also raises concern that the associations are not reflecting the effects of the measured pollutants, but rather some factor or combination of factors, such as, for example, unmeasured air pollutants or uncontrolled features of meteorology that are correlated with the measured pollutants."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitors Was the exposure concentration measured? (+) 3 years Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (n/a no symptom data) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported?

	(+) Did the investigator appropriately interpret the statistics? (few) Are the observed trends statistically significant?
G. Interpretations:	(objective no clearly stated) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (more detail needed) Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)?(No specific SO_2 conclusions) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The results for SO_2 and mortality were inconsistent. The percentage increases in mortality were relatively small and the confidence intervals were wide. Perhaps the stratification by season resulted in a lack of statistical power, or the extensive smoothing of the data affected the associations.

The large number of observations available enhanced the ability of this study to assess effects at the low end of the concentration-response relationship. While conclusions from this study support the argument that there are no threshold concentrations of air pollution below which adverse effects cannot be detected, it also raises the concern that some confounding factor, such as unmeasured air pollutants, was not being reflected by the study.

Confidence index:

Some confounders not considered such as influenza epidemics.

Stratification of the data by season may have resulted in a lack of statistical power.

The objective is not clearly stated.

The variability between the SO_2 monitoring sites is not discussed.

Good explanation of estimation of missing SO_2data.

The majority of the paper focuses on the PM_{10} results.

This study did not provide substantial information on the subjects involved in the study. Cause of death and date of death was included, but possible confounding factors such as socioeconomic status and previous health history were not included.

Confidence intervals were not reported in this study. For missing hourly data, values were filled in for each pollutant and each meteorologic variable using the EM algorithm.

A limitation of this study is the exposure assessment as it is difficult to monitor how or when individual subjects are exposed.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Peters, A., Goldstein, I.F., Beyer, U., Franke, K., Heinrich, J., Dockery, D.W., Spengler, J.D., Wichmann, H.E.	**Internal ID:** 435

Title: Acute Health Effects of Exposure to High Levels of Air Pollution in Eastern Europe

Year: 1996

Abstract: Acute effects of winter-type air pollution characterized by high levels of SO_2, moderate levels of particles, and low acidity were studied. A panel of 155 asthmatic children and 102 adults with a history of asthma from the former German Democratic Republic cities of Erfurt and Weimar and from the Czech Republic city of Sokolov participated from September 1990 through June 1992. The panelists recorded daily symptoms, medication intake, and peak expiratory flow (PEF). Statistical analysis was based on linear regression of population-averaged time series controlling for trend, meteorology, and autocorrelation. A temporospatial time series approach was also applied to the data to eliminate possible confounding by some known or unknown variables that occurred simultaneously in two of the study areas. Weak same-day effects and a stronger cumulative effect of air pollution on children was observed both for PEF and for symptoms. PEF decreased -0.90% (-1.35 to -0.46%), and a symptom score increased 14.7% (0.8-28.6%) in association with an average increase of 128 $\mu g/m^3$ SO_2 over the previous 5 days. Effects on adults were smaller and less consistent. Morbidity of children was best predicted by SO_2 and sulfate concentrations. The authors conclude that prolonged, high exposure to winter-type pollution was associated with small adverse health effects in asthmatics.

Objective: In this paper, we report on the first panel study to assess acute health effects of air pollution conducted in Eastern Europe.

Overall study design:

Exposure level	**Exposure frequency/ duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
WINTER 1990-1991 Erfut: Mean-125 $\mu g/m^3$ Max-564$\mu g/m^3$ Interquartile Range (IQR)-113$\mu g/m^3$ Weimar: Mean-236 $\mu g/m^3$ Max-1018$\mu g/m^3$ Interquartile Range (IQR)-207$\mu g/m^3$ Sokolov:	Study period – 1990 to 1992	For the 3 cities: Children: female 24%-54% Adults: female 47%-67%	102 adults: 32-80y 155 children: 7-15y	257	Asthmatic	n/a	n/a

Mean-90 $\mu g/m^3$ Max-492$\mu g/m^3$ Interquartile Range (IQR)-94$\mu g/m^3$ WINTER 1991-1992 Erfut: Mean-96 $\mu g/m^3$ Max-462$\mu g/m^3$ Interquartile Range (IQR)-80$\mu g/m^3$ Weimar: Mean-153 $\mu g/m^3$ Max-794$\mu g/m^3$ Interquartile Range (IQR)-130$\mu g/m^3$ Sokolov: Mean-71 $\mu g/m^3$ Max-383$\mu g/m^3$ Interquartile Range (IQR)-66$\mu g/m^3$							

Observations:

For an increase in SO_2 equal to an IQR of 133$\mu g/m^3$, weak associations between decreases in peak expiratory flow in the evening before medication use and increased levels of SO_2 on the same day were associated for children; however, the association was not statistically significant (combined analysis of all cities).
Stronger associations were obtained for 5-day mean concentrations of SO_2 and peak expiratory flow in children (not statistically significant).
Effects on adults were small and considerably more variable.
SO_2 on the current day showed no relation to the symptom score on the same day although symptoms were associated with a 5-day mean of SO_2 (not statistically significant).

Separate effect estimates for the three panels and two winters showed a considerable variability.
Effects on adults were small and considerably more variable.
PEF decreased -0.90% and a symptom score increased 14.7% in association with an average increase of 128 μg/m^3 SO_2 over the previous 5 days. Morbidity of children was best predicted by SO_2 and sulfate concentrations.
Levels of SO_2 and TSP differed in the three locations. The highest SO_2 concentrations were measured in Weimar, where the current WHO air quality standard of 125 μg/m^3 was violated 145 times in the first winter and 105 times in the second winter. SO_2 concentrations were notably lower in Erfurt and still lower in Sokolov. The correlation between SO_2 and TSP was 0.8-0.9 in Erfurt during both winters and in Weimar during the first winter. The low correlation coefficients between SO_2 and SP in Sokolov and in Weimar during the second winter reflected the different patterns for the two pollutants. Associations between SO_2 and PEF appeared to be stronger in the second winter; increased symptoms were more often reported during prolonged SO_2 episodes in the first winter. 5-day mean concentrations of SO_2, PM_{10}, and particle strong acidity were related to an increased number of symptoms. Associations were found to be statistically significant only between SO_2 and PEF during the winter.

Author's Conclusions:

The authors conclude that prolonged, high exposure to winter-type pollution was associated with small adverse health effects in asthmatics
"In an atmosphere with high SO_2 concentrations, small decreases of lung function and increases in symptoms were observed in association with SO_2 and sulfate concentrations of fine particles for children, with still weaker effects for adults. We wish to emphasize that these results may not be generalizable to other panes of subjects with asthma. It is important to remember that the mix of pollutants is very different from the usual mix of pollutants present in Western countries, particularly the United states, where many of the previous studies have been carried out. Given the high concentrations of SO_2 in our study area, levels of PM_{10} sulfates and acidity in particular were surprisingly low. Oxidation of SO_2 might have been incomplete, and neutralization by ammonia appeared to play an important role."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(more info needed)** Is the number of subjects sufficient to produce statistically significant results? **(ok)** Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(-) more info needed** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? **(more info needed)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	**(+) fixed monitors** Was the exposure concentration measured? **(+) study period 1990-1992** Is the exposure duration known?
C. Equipment:	**(-)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	**(-)** Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval?

	(n/a no controls) Were appropriate controls recruited? (no) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) more detail needed Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (-) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+/-) Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (+) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (few) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (more detail needed) Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

A small decrease in pulmonary function and a small increase in reported symptom score associated with air pollution were observed in children with asthma in this study. These effects became more pronounced when a 5 day mean was used to represent cumulative effect of exposure to air pollution. The effects were small and inconsistent in adults. When the city data was examined separately, the effects estimates were highly variable.

The authors conclude that prolonged high exposure to winter-type pollution was associated with small adverse health effects in asthmatics. The role air pollution plays in asthma in Eastern Europe remains an open question. Cross-sectional studies showed the prevalence of respiratory symptoms to be elevated in the more highly polluted East German cities. Air pollution has not been shown to be of primary importance in affecting the prevalence of asthma in these studies. Similar results have been found comparing an urban region in Poland with rural and urban regions in Sweden. These findings indicated that air pollution was not a primary risk factor for the development of asthma.

Confidence index:

The levels of SO_2 were highly variable between the cities.
Only 1 central monitoring station per city.
More detail is needed to determine whether sample size is large enough.
More information is needed on the recruitment of subjects.
The data collected in each city was collected over different months.
The follow-up times varied between patients.
Different symptoms questionnaires were used for children and adults.
Most of the subjects used medication and were lifelong asthmatics. They may be less susceptible to the air pollution due to better management of illness.
Good detail on statistical analysis.
The combined analysis is questionable due to the differences between cities.
The severity of asthma and the medical treatment of the disease did differ between the locations.
This study clearly described the recruitment process and described why some individuals had to be excluded from the study. A basic questionnaire, administered at the beginning of the study, enabled collection of data regarding their history of respiratory disease.
No control group was recruited in this study. Monitoring device information was not given, and raw and individual data were not reported.
Most of the focus in this paper was on SO_2 rather than groups of pollutants.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Peters, A., Doring, A., Wichmann, H.E., Koenig, W.	**Internal ID:** 436
Title: Increased plasma viscosity during an air pollution episode: a link to mortality	
Year: 1997	
Abstract: BACKGROUND: Air pollution episodes have been consistently associated with increased mortality, and most strikingly with mortality due to cardiovascular disease. One hypothesis to explain this association is that inflammation of the peripheral airways caused by pollution might increase blood coagulability. We have tested this hypothesis in a cross-sectional study by comparing measurements of plasma viscosity during a severe episode of air pollution during 1985 with those made on less polluted days. METHODS: Plasma viscosity was measured as part of the MONICA Augsburg survey during the winter of 1984-85 in 3256 randomly selected men and women aged 25-64 years. Daily mean concentrations of air pollutants and meteorological variables were measured in Augsburg as part of the automated Bavarian air-quality network. We compared measurements of plasma viscosity made in 324 people who attended for screening during the pollution episode and in 2932 people screened during the remainder of the survey period. FINDINGS: In January, 1985, high concentrations of sulphur dioxide (mean 200 $\mu g/m^3$) and total suspended particles (mean 98 $\mu g/m^3$) were recorded during a 13-day period in Augsburg. In men, the odds ratio for plasma viscosity above the 95th percentile of the distribution (1.38 mPa s) was 3.6 (95% CI 1.6-8.1) comparing measurements during the air pollution episode with non-episode measurements after adjustment for cardiovascular risk factors and meteorological variables. The corresponding odds ratio for women (95th percentile of plasma viscosity 1.37 mPa s) was 2.3 (1.0-5.3). High concentrations of carbon monoxide were also associated with increased plasma viscosity in women. INTERPRETATION: During the 1985 air pollution episode, an increased risk of extreme values of plasma viscosity was observed in both men and women. Altered blood rheology due to inflammatory processes in the lung that induce an acute-phase reaction might therefore be part of the pathological mechanisms linking air pollution to mortality.	
Objective: The analyses presented here focus on the question: was plasma viscosity higher than normal in a random sample of the population in Augsburg during the days of the 1985 air pollution episode.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean: Pollution episode Jan7-19,1985 $200.3 \pm 8.4 \mu g/m^3$ Range: 160-$238 \mu g/m^3$ Remainder of period: Mean:$48.2 \pm 2.1 \mu g/m^3$ Range: $13\text{-}103 \mu g/m^3$	Study period Oct 1984-May1985 144 days	Men-1663 Women-1593	25-64 y	3256	No info	n/a	n/a

Observations:

Plasma viscosity was on average 0.013mPa s higher on episode days than on nonepisode days in men (p=0.015). There was a difference of 0.017 mPa s in plasma viscosity between episode days and non-episode days in women (p=0.002). For plasma viscosity above the 95th percentile, after adjusting for cardiovascular risk factors and meteorology, the OR was 3.62 (95% CI 1.6-8.1) and 2.26 (95% CI 1.0-5.3) for men and women respectively. However, the confidence intervals were very wide.

For the rest of the study period, the associations between plasma viscosities above the 95th percentile for an increase of $165\mu g/m^3$ were not significant for men. For women, the OR varied from 2.49 (current day exposure) to 2.56 (lag 3) but the confidence intervals were very wide.

High concentrations of CO were also associated with increased plasma viscosity in women.

*Statistically significant

Author's Conclusions:

"Our findings suggest that plasma viscosity may be an intermediate step for both particulate air pollution and carbon monoxide, which have been found to be associated with hospital admission for cardiovascular disease."

"During the 1985 air pollution episode, an increased risk of extreme values of plasma viscosity was observed in both men and women. Altered blood rheology due to inflammatory processes in the lung that induce an acute-phase reaction might therefore be part of the pathological mechanisms linking air pollution to mortality."

"Models for long-range transport of air pollution suggest that sulphur dioxide and respirable particulate matter had been transported to Augsburg in addition to local sources. Later in January, 1985, the same air masses originating from the industrial centres of the former German Democratic Republic, where sulphur-rich coal had been used, reaching the Ruhr area, the Netherlands, and the UK, and much higher values of air pollutants were recorded. Evidence for inflammatory responses to sulphur dioxide and particulate air pollution has been found in experiments in animals.

The increased plasma viscosity observed in the analyses of a cross-sectional survey might therefore represent a part of the pathophysiological chain linking high ambient air pollution to increased mortality and hospital admissions for cardiovascular diseases."

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A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (more details needed) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure	(+) fixed monitors Was the exposure concentration measured?

conditions:	(+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no control)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) **cross sectional study** Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) ((+) Were accepted assessment methods employed? (more detail needed) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (more details needed) Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (-) Do the conclusions make biological sense? (-) Are the extrapolations reasonable?

	(+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Plasma viscosity increased in men and women during the pollution episode. However, the results were not analyzed for particular concentration of SO_2 during this episode. A cross-sectional study was performed and plasma viscosity during a severe episode of air pollution was compared with those made on less polluted days. There is too much variability to confidently conclude that the increased plasma viscosity is due to SO_2.

Findings suggest that plasma viscosity may be an intermediate step for both particulate air pollution and carbon monoxide, which have been found to be associated with hospital admissions for cardiovascular diseases.

Confidence index:

More detail is needed on the recruitment of the subjects.

More detail needed on the plasma analysis.

Confounders and other risk factors were considered but hard to control for in this cross-sectional study.

A close correlation between individual cardiovascular risk factors and the coincidence of low temperatures and high air pollutant concentrations might have resulted in some variation of the effect estimates for men and women.

Results indicated that a particular group of individuals in the population of Augsburg might be more susceptible.

The study recorded height, bodyweight, body-mass index and smoking behaviour - variables that could act as confounding variables. Furthermore, the authors recorded meteorological variables that could interfere with interpretation of findings. However, in this type of study, it is difficult to limit confounding variables.

A strong limitation in this study is exposure assessment. Blood samples were taken from randomly selected individuals; however, it is difficult to quantify the subjects' exposure.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Sunyer, J., Saez, M., Murillo, C., Castellsague, J., Martinez, F., Anto, J.M.	**Internal ID:** 437

Title: Air Pollution and Emergency RoomAdmissions for Chronic Obstructive Pulmonary Disease: A 5 year study.

Year: 1993

Abstract: An association between sulfur dioxide levels in urban air and the daily number of emergency room admissions for chronic obstructive pulmonary disease was previously reported in Barcelona, Spain, for the period 1985-1986. The present study assesses this association over a longer period of time, 1985-1989. This made it possible to carry out separate analyses for the winter and summer seasons and thus to control more adequately for weather and influenza epidemics. An increase of 25 μg/m^3 in sulfur dioxide (24-hour average) produced adjusted changes of 6% and 9% in emergency room admissions for chronic obstructive pulmonary disease during winter and summer, respectively. For black smoke, a similar change was found during winter, although the change was smaller in summer. The association of each pollutant with chronic obstructive pulmonary disease admissions remained significant after control for the other pollutant. The present findings support the conclusion that current levels of sulfur dioxide and black smoke may have an effect on the respiratory health of susceptible persons.

Objective: The present study assesses the association between the daily number of emergency room admissions for COPD and daily levels of sulfur dioxide and black smoke in the air of Barcelona during the period 1985-1989. A second objective was to estimate whether the association of COPD with sulfur dioxide is independent of the role of black smoke.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean levels not provided Quartile Cutpoints: Winter : Q1-36μg/m^3; Q2-49μg/m^3; Q3-67μg/m^3 Summer: Q1-20μg/m^3; Q2-29μg/m^3; Q3-40μg/m^3	1985-1989	No info	No info	Daily mean number of emergency room admissions for COPD was 15.8 in winter and 8.3 in summer.	No info	n/a	n/a

Observations:

Sulfur dioxide was positively associated with COPD emergencies in winter and summer. A significant association was seen for sulfur dioxide levels in both seasons after controlling for confounders (autocorrelation, seasonality, and meteorologic conditions).
The 1 day lagged values resulted in slightly lower associations; the associations disappeared with 2 day lagged values.
An increase of 25µg/m^3 in sulfur dioxide produced an adjusted change of approximately 6 % in the number of COPD emergencies during winter and an adjusted change of 9 % in summer.
An adjusted positive relation of SO_2 with COPD emergencies was found in each tertile of black smoke during both seasons. An adjusted relation of black smoke with COPD admissions was found across strata of SO_2 in the winter. After inclusion of SO_2 and black smoke in the same ridge regression model, both pollutants showed a similar adjusted association with the daily number of COPD emergencies during winter, but only SO_2 did so during summer.

*Statistically significant

Author's Conclusions:

"The present findings support the conclusion that current levels of sulfur dioxide and black smoke may have an effect on the respiratory health of susceptible persons."
"Sulfur dioxide might be an indicator of other nonstudied pollutants, such as particles smaller than 10um, sulfates and acid aerosols, or even a mixture of pollutants. However, using the available data on sulfates, we observed that sulfur dioxide was still related to COPD emergencies after we controlled for sulfates. We found the same thing after controlling for nitrogen dioxide and ozone.
Although concentrations of sulfur dioxide and black smoke have been reduced in Barcelona as well as in other developed countries, current levels of sulfur dioxide and black smoke might still impair the health of vulnerable persons such as those suffering from chronic respiratory disease."

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A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitors Was the exposure concentration measured? (+) Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited?

	(n/a no blinding) Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (more details needed) Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.,* additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (+) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Sulfur dioxide and black smoke concentrations in the urban air of Barcelona were associated with the number of COPD emergency room admissions in both winter and summer. An increase of 25μg/m^3 in sulfur dioxide produced adjusted changes of 6% and 9% in emergency room admissions for chronic obstructive pulmonary disease during winter and summer, respectively. Even at low concentrations, SO_2 exposure might still impair the health of vulnerable persons and those suffering from chronic respiratory disease.
This study was built on a previous study that found a relation between sulfur dioxide and the daily number of emergency room admissions for chronic obstructive pulmonary disease in Barcelona.
Confidence index:
Much of the methodology detail is reported in another study.
Fifteen manual samplers were used to measure SO_2. The mean level of sulfur dioxide measured by the manual samplers was correlated with sulfur dioxide analyzed by molecular fluorescence from an automatic station located in the center of the city.
Several confounders were controlled in the analysis.
Average levels of SO_2 not provided. Details included in a previous study.
More detail needed in results section.
Because this study was concerned only with obtaining data from emergency registries, no recruitment methods were necessary. The registries can only provide limited personal data, so socioeconomic information was not available for the subjects involved. Unlike other studies, the large series of data permitted the authors to do studies over the winter and over the summer. Exposure assessment is a weakness here as confounding variables such as additional chemical exposure were not limited.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Sunyer, J., Anto, J.M., Murillo, C., Saez, M.	**Internal ID:** 439

Title: Effects of Urban Air Pollution on Emergency Room Admissions for Chronic Obstructive Pulmonary Disease

Year: 1991

Abstract: The authors assessed the relation between sulfur dioxide and other air pollutants (black smoke, carbon monoxide, nitrogen dioxide, and ozone) and daily emergency room admissions for chronic obstructive pulmonary disease in Barcelona, Spain, during 1985-1986. Barcelona is a Mediterranean city of 1.7 million inhabitants that has air pollution levels lower than standard values. A weak but statistically significant association between emergency room admissions and levels of sulfur dioxide, black smoke, and carbon monoxide was observed. Daily emergency room admissions for chronic obstructive pulmonary disease increased by 0.02 and 0.01 for each microgram of sulfur dioxide and black smoke per cubic meter, respectively, and 0.11 for each milligram of carbon monoxide per cubic meter, after adjusting for meteorologic and temporal variables. Similar estimations were obtained after controlling for the autocorrelation effect by means of time series analysis. In addition, when the data were stratified by season, the effects remained in summer. This strengthened the conclusion that the relation observed in winter, spring, and throughout the study period was indeed real. After truncating the data according to sulfur dioxide or black smoke levels, the authors still found effects for these pollutants at levels below the air quality guideline of 100 μg/m^3 established by the World Health Organization. The consistency of these results with other recent toxicologic and epidemiologic observations suggest that current air pollution standards do not totally protect public health with a margin of safety in specific places.

Objective: We designed a study to assess the relation between levels of sulfur dioxide and other air pollutants and emergency room admissions for chronic obstructive pulmonary disease.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
24-hour Average: Yearly mean: 56.5±22.5μg/m^3 Range: 17-160 μg/m^3 1-hour Maximum Value: Yearly mean: 141.9±98.8 μg/m^3 Range:14-720 μg/m^3	1985-1986	Male and female	>14 y 99.4 percent of admissions corresponded to period over age 35 y)	Daily mean number of emergency room admissions for chronic obstructive pulmonary disease was 11.9±5.6 (Admissions information obtained from register of respiratory emergencies)	Chronic Obstructive pulmonary disease	n/a	n/a

Observations:

A statistically significant relation was found between sulfur dioxide and emergency room admissions for chronic obstructive pulmonary disease:

Daily air pollutant mean values according to the daily number of emergency room admissions for chronic obstructive pulmonary disease (COPD),

Barcelona, Spain, 1985-1986 (pvalue <0.001)

Air Pollutant Mean Values

COPD by Quartile	24hour average $SO_2(\mu g/m^3)$	1hour maximum value $SO_2(\mu g/m^3)$
≤8	48	115.5
9-11	54.5	128.6
12-14	57.3	140.2
>14	64.4	171.1

When stratifying for temperature, the relation between emergency room admissions for chronic obstructive pulmonary disease and sulfur dioxide was stronger in the highest temperature stratum:

Sulfur dioxide 24-hour average mean values according to the daily number of emergency room admissions for COPD and minimum temperature (°C), Barcleona, Spain, 1985-1986

	Sulfur dioxide$(\mu g/m^3)$	
COPD by Quartile	Temperature	
	≤11.7	>11.7
≤8	60.3	47.5
9-11	61.0	53.6
12-14	59.1	56.9
>14	69.4	61.1
pvalue	**(0.09)**	**(<0.001)**

Sulfur dioxide (24 hour average) was significantly related to emergency room admissions for COPD after adjusting for meteorological and temporal variables ($p<0.01$). An adjusted change of 0.50 daily emergency room admissions for chronic obstructive pulmonary disease per unit change of an approximate increase of 25 $\mu g/m^3$ SO_2 was found. This amount of change in daily admissions was the same as that found for sulfur dioxide measured as one-hour maximum value. This relation remained unchanged when sulfur dioxide values were substituted by their one-day lagged values. However, when substituted for lag 2 days, a lower and nonstatistically significant effect was found.

After stratification by season, sulfur dioxide had a crude positive relation with emergency room admissions for COPD in all seasons which were all statistically significant.

	Sulfur Dioxide ($\mu g/m^3$)			
Quartile of COPD*	Winter	Spring	Summer	Fall
1	56.4	46.8	41.2	47.1
2	60.2	48.0	50.9	59.9
3	69.6	54.7	56.0	56.2
4	64.8	56.7	60.3	64.1
pvalue	0.04	0.04	<0.01	0.03

*The quartile limits of the COPD distribution were different according to each season. These were for winter, 14, 17 and 20;spring, 8, 10 and 13; summer 6, 8 and 11; and fall, 7, 11, and 14.

At 24 hour average levels less than 100 $\mu g/m^3$, there were statistically significant effects for sulfur dioxide for emergency room admissions for COPD. For 150 $\mu g/m^3$, the increase in admissions was 0.55/day ($p<0.01$), for 100 $\mu g/m^3$, the increase in admissions was 0.70/day ($p<0.01$) and for 72 $\mu g/m^3$, the increase in admission was 0.70/day ($p=0.04$). (The daily admissions in this case have been adjusted by minimum temperature lagged by 2 days, wind velocity lagged by one day, season, and day of the week.)

A weak but statistically significant association between emergency room admissions and levels of black smoke and carbon monoxide was observed. Daily emergency room admissions for chronic obstructive pulmonary disease increased by 0.02 and 0.01 for each microgram of black smoke per cubic meter, respectively and 0.11 for each milligram of carbon monoxide per cubic meter, after adjusting for meteorologic and temporal variables. Similar estimations were obtained after controlling for the autocorrelation effect by means of time series analysis. In addition, when the data were stratified by season, the effects remained in summer. This strengthened the conclusion that the relation observed in winter, spring, and throughout the study period was indeed real. After truncating the data according to sulfur dioxide or black smoke levels, the authors still found effects for these pollutants at levels below the air quality guideline of 100 $\mu g/m^3$ established by the World Health Organization.

Author's Conclusions:

"From the point of view of the etiology of the exacerbation of chronic obstructive pulmonary disease, the role of air pollutants at current levels in Barcelona appears to be of little relevance, since the strength of the association was weak.

From a wider point of view, the effects of air pollution on emergency room admissions for COPD can be considered as evidence of a biological impact with a potential effect on health apart from respiratory diseases. Thus, the present findings provide information relevant to the evaluation of air pollution standards and guidelines.

The consistency of these results with other recent toxicological and epidemiologic observations suggest that current air pollution standards do not totally protect public health with a margin of safety in specific place."

"A major finding was that sulfur dioxide and black smoke effects on emergency room admissions for chronic obstructive pulmonary disease appeared at lower levels than the corresponding European Community standards, and even at levels below the lower World Health Organization air quality guidelines. Air quality guidelines and standards indicate levels at which no adverse health effects are expected, therefore the present results indicate that current air quality standards do not fully protect the health of some population groups."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from

guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(No information on total admissions)** Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)**Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **Fixed monitoring sites**Was the exposure concentration measured? (+) **2 year study period**Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? **(No info)**What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)**Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)**Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(Not enough info)**Were accepted assessment methods employed? (+/-)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (-)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?

G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

A positive and statistically significant relationship was found between daily number of emergency room admissions for chronic obstructive pulmonary disease and daily levels of sulphur dioxide. The magnitude of the observed effects was weak (an increase of 100μg/m^3 of sulfur dioxide daily mean led, on average, to an increase of two emergencies per day for COPD). The study design is not strong enough to make the claim that current air pollution standards do not totally protect public health.

The daily mean number of emergency room admissions for COPD was 11.9. A positive and statistically significant relation was found between daily number of emergency room admissions for COPD and daily levels of SO_2, BS, and CO. The magnitude of the observed air pollution effect on emergency room admissions for COPD was weak.

Confidence index:

Insufficient literature review in introduction.

The objective states that the relationship between air pollutants and emergency room admissions for COPD will be studied. However, in the discussion, the authors discuss the short-term causal relationship though this is an ecological study.

To account for differences in terminology a panel of chest physicians drew up a list of expressions used that would qualify for inclusion as chronic obstructive pulmonary disease. This may have resulted in misclassification or an overestimate of the admissions.

Approximately 20% of the pollutant monitoring data was missing due to malfunction of equipment.

Most of the results were just weakly significant.

Authors state that even though the magnitude of the effects was small, they should not exist at all because it occurred at levels below European Community standard values and WHO guidelines. However, they do no consider that this very weak association could be due to misclassification or chance, confounding variables,etc.

According to the authors, the diagnosis of emergency room admissions for chronic obstructive pulmonary disease in emergency room services has not been validated.

City wide average levels of pollution instead of a population weighted city average were used.

Statistical analysis was well explained with listing of assumptions and variables of various statistical models.
The process for the selection of confounder variables to include in the models was well explained.
Data was examined for the whole year and seasonally.
Misclassification between emergency room admissions for COPD and asthma was considered in the statistical models.
The study considered both 24-hour daily averages and one hour maximum values.
Report consistent with other toxicological and epidemiological studies.
This paper made reference to other resources for details of the study design. However, information such as age, gender and health history were provided.
Confidence intervals were not reported.
Raw and individual data were not included

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Bobak, Martin; Leon, David A.	**Internal ID:** 440
Title: Air Pollution and infant mortality in the Czech Republic, 1986-88	

Year: 1992

Abstract: An ecological study of infant mortality and air pollution was conducted in the **Czech** Republic. Routinely collected data on infant mortality and air pollution in the period 1986-88 were analyzed for the 46 of the 85 districts in the republic for which both were available. The independent effects of total suspended particulates (TSP-10), sulphur dioxide (SO_2), and oxides of nitrogen (NO_x) adjusted for district socioeconomic characteristics, such as income, car ownership, and abortion rate, were estimated by logistic regression. We found weak positive associations between neonatal mortality and quintile of TSP-10 and SO_2. Stronger adjusted effects were seen for postneonatal mortality, with a consistent increase in risk from the lowest to the highest TSP-10 quintile ($p < 0.001$). Weaker and less consistent evidence of a positive association with NO_x ($p = 0.061$) was observed. The strongest effects were seen for postneonatal respiratory mortality, which increased consistently from lowest to highest TSP-10 quintile ($p = 0.013$). There was also a suggestion of a positive association with SO_2 ($p = 0.062$). The highest to lowest quintile risk ratios for postneonatal respiratory mortality were 2.41 (95% CI 1.10-5.28) for TSP-10, 3.91 (0.90-16.9) for SO_2, and 1.20 (0.37-3.91) NO_x. The specificity of the association between air pollution quintile (especially TSP-10) and postneonatal respiratory mortality is consistent with the known effects of air pollution on respiratory disease morbidity in children. These ecological associations require confirmation in an individually based study.

Objective: We have studied the strength of any association between air pollution within the Czech Republic and infant mortality, focusing on the extent to which different components of air pollution have independent effects upon mortality in the neonatal and postneonatal periods.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	**Pre-trial health**	**NOAEL**	**LOAEL**
Mean – 31.9 $\mu g/m^3$ (Range $<12.5\mu g/m^3$ - $>57.9\mu g/m^3$)	Continuous (fixed monitoring stations)/1986-1988	Male and female	Infant	Out of approximately 222,000 live births: 1819 deaths (neonatal period-less than one month) 880 deaths (postnatal period-1month to 1 year)	**n/a**	**n/a**	**n/a**

Observations:

Weak positive associations were found between neonatal mortality and quintile of TSP-10 and SO_2. Stronger adjusted effects were seen for postneonatal mortality, with consistent increase in risk from the lowest to the highest TSP10 quintile. There was also a suggestion of a positive association with SO_2. The highest to lowest quintile risk ratios for post neonatal respiratory mortality was 3.91 for SO_2. The respiratory disease (post neonatal respiratory mortality) linear trend statistics for SO_2 are significant at the 5% level.

Author's Conclusions:

"The associations between air pollution and infant mortality seen in this study are relatively specific. Although there is a suggestion of weak effects on neonatal mortality and postneonatal non-respiratory mortality much more substantial effects are observed for postneonatal respiratory mortality. These effects are seen principally for TSP-10 and SO_2. Postneonatal respiratory mortality increases consistently from the lowest to the highest TSP-10 quintile, an effect that is independent of that of either of the other two measured pollutants."

"There was a suggestion of a positive association with SO_2 ($p=0.062$)."

"The specificity of the association between air pollution quintile and postneonatal respiratory mortality is consistent with the known effects of air pollution on respiratory disease morbidity in children."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a) Were identical 'criteria for exclusion' applied to both cases and controls? (n/a) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (-)Is the exposure duration known?
C. Equipment:	(+)Was a full description of the monitoring devices provided? (No information) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (-)Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a) Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (n/a)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive?

	(n/a)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (-)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a) Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings in this study were able to show a statistically significant association between SO_2 and infant mortality. There was one statistically significant result when SO_2 concentrations were related to postneonatal respiratory mortality. The adjusted (for confounders) relative risks were high; however the confidence intervals were large. (Eg. RR=5.41 Range=1.75-16.8). Acknowledging the inherent weaknesses in an ecological study, this study fails to give sufficient evidence to generate a hypothesis regarding infant mortality and SO_2 exposure.

Confidence index:

The literature review was inadequate. The introduction didn't give much detail on the planning of the research.
Some confounders were considered; however the seasonality of the data was not. There was no justification for some of the confounders considered, i.e. mean number of persons per car. Because of the ecological design, data on smoking, indoor air pollution and other family circumstances is not available.
The area over which the air pollution was measured would not necessarily result in homogenous exposures. The districts were grouped by level of pollutant

concentrations; however, each district has varying terrain, socioeconomic status, etc.

The districts chosen for monitoring were not chosen randomly. Only districts with monitoring stations were chosen. No data was available for the non-monitored districts. Included sampling districts have higher levels of air pollution and heavier concentrations of manufacturing industry than excluded districts possibly leading to skewed results.

Very limited information is provided in the data and methods section.

Most relative risks are less than 2 and the confidence intervals include RR=1.

Neonatal and postneonatal mortality and postneonatal respiratory mortality are all examined. The author's give one code ICD-9 for classification of death but don't give details on how postneonatal and postneonatal respiratory were differentiated. There is no information on whether the respiratory disease was present at birth.

Increases in SO_2 concentration did not result in a larger number of deaths and time lags were not considered.

The author's report a J-shaped relationship between SO_2 and infant mortality, but provide no details or insight.

The discussion includes very few references and only compares the present study with one other study that looked at air pollution and morbidity and mortality in children.

The results cannot be generalized to the whole population.

There is no information on the statistical reliability of the routinely collected pollution data.

The study had several weaknesses, including the limitations of ecological studies. In addition, the districts included in the study were those in which routine air pollution data was unavailable. Furthermore, this study did not adequately adjust for socioeconomic factors, indoor pollution and family size.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Peters A., Emerson, L., Verrier, R.L., Schwartz, J., Gold, D.R., Mittleman, M., Baliff, J., Oh, J.A., Allen, G., Monahan, K., Dockery, D.W.	**Internal ID:** 441

Title: Air Pollution and Incidence of Cardiac Arrhythmia

Year: 2000

Abstract: Air pollution episodes have been associated with increased cardiovascular hospital admissions and mortality in time-series studies. We tested the hypothesis that patients with implanted cardioverter defibrillators experience potentially life-threatening arrhythmias after such air pollution episodes. We compared defibrillator discharge interventions among 100 patients with such devices in eastern Massachusetts, according to variations in concentrations of particulate matter, black carbon, and gaseous air pollutants that were measured daily for the years 1995 through 1997. A 26-ppb increase in nitrogen dioxide was associated with increased defibrillator interventions 2 days later (odds ratio = 1.8; 95% confidence interval = 1.1-2.9). Patients with ten or more interventions experienced increased arrhythmias in association with nitrogen dioxide, carbon monoxide, black carbon, and fine particle mass. These results suggest that elevated levels air pollutants are associated with potentially life-threatening arrhythmia leading to therapeutic interventions by an implanted cardioverter defibrillator.

Objective: We tested the hypothesis that patients with a history of serous arrhythmia requiring implanted cardioverter defibrillators (ICDs) would experience potentially life threatening arrhythmia associated with air pollution episodes.
We report the results of a pilot study to assess the feasibility of linking cardiac arrhythmias detected by ICD devices with air pollution.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Measurements from fixed monitoring station: Mean = 0.007 ppm	Hourly measurements taken Jan 1995-Dec 1997 63,628 person-days of follow-up over this 3yr period.	79 female 21 male	Mean 62.2 yrs Range 22-85	100	History of serious arrhythmia	n/a	n/a

Observations:

Season was a strong predictor of the defibrillator discharges, with the highest frequency during the summer months and a second peak during the second half of the winter.
No consistent evidence of increased defibrillator discharges associated with the concentration of the SO_2 on the same day for the sample of patients with discharges.
No association was observed between the defibrillator discharges and SO_2.

(Statistical significance of any results was not reported)
The study population was predominantly male with a mean age of 62.2 years. During 63,628 person days of follow-up over 3 years in 100 patients, 223 defibrillator discharges were observed. SO_2 and O_3 were weakly correlated with the other pollutants, suggesting different seasonal patterns and sources. The highest $PM_{2.5}$, PM_{10}, O_3, and SO_2 concentrations were recorded during the summer, whereas black carbon, CO, and NO_2 had elevated peak concentrations throughout the year. Patients with 10 or more interventions experienced increased arrhythmias in association with nitrogen dioxide, carbon monoxide, black carbon, and fine particle mass.

Author's Conclusions:

The authors make no specific conclusions with regards to SO_2 exposure and defibrillator discharges. This is probably due to the lack of a strong association between the two variables.
"The subgroup of patients with repeated potentially life-threatening arrhythmias was most susceptible to exposure to ambient air pollution. Repeated discharges indicate that these patients belong to a subgroup of patients who experience acute arrhythmia in response to triggers. This subgroup might be especially sensitive to air pollution. This group also provides most of the power for the analyses, because other potent triggers, such as transient ischemia, add noise to the association o interest. Therefore, we would be more likely to detect an air pollution association in patients with repeated events than in patients who only experience one or two events during the 3-year follow up."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a)Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (+ measurements taken over 3 yr period) Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (No information) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (-)Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)

E. Data collection:	(n/a) Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+/-) Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-) but general inclusion criteria given Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (significance not given) Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a – no specific conclusions re SO_2 given) Do the conclusions make biological sense? (n/a) Are the extrapolations reasonable? (yes)Is the paper in a peer-reviewed journal? (no)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Increased risk of cardiac arrhythmia (using ICD devices for detection) in association with elevated concentrations of SO_2 was not observed in this study. Out of the 100 cases, only 33% suffered any events over the three year period, significantly decreasing the sample size. Also, of the 33 cases with at least one cardiac event, 6 of the cases appeared to be significantly more sensitive than the rest indicating differences among the subjects. This cohort may be of special interest because their previous history of cardiovascular disease might make them particularly sensitive to the effects of air pollution episodes. However, data on baseline clinical characteristics and prescribed antiarrhythmic medications were not available in this pilot study to determine the characteristics associated with increased (or decreased) responsiveness.

An increased risk of cardiac arrhythmia was observed in association with elevated concentrations of air pollutants in patients with ICDs. Authors found support for the hypothesis that $PM_{2.5}$ would be associated with the incidence of defibrillator discharges. A stronger association was found for NO_2 and black carbon than for $PM_{2.5}$.

Confidence index:

The objective and hypothesis of the study were clear; however, there is no clear statement in the conclusion on whether the author's conclude that the cardioverter is a feasible way of linking cardiac events detected by ICD devices with air pollution exposures.

The use of the cardioverter defibrillator may be an effective way of measuring the cardiac events caused by SO_2 exposure, providing unbiased objective measurements. However, there is no way of determining the exact cause of the cardiac event or the nature of the event, as no clinical data were available for this study. As pointed out by the authors, the ICD devices are optimized to avoid underdetected ventricular tachyarrhythmia which may result in detecting events produced by normal activities.

There appeared to be much variability in the level of illness in the cases. Out of the 100 cases chosen, only 33 experienced a cardiac event in 3 years and 6 of the subjects were much more sensitive than the rest, experiencing over 10 events in the 3 years. The analyses could only be performed for the 33 subjects. The data are likely not generalizable to the entire population.

Age and gender were reported, but confounding factors were not considered. However, it was reported that of the particularly sensitive subgroup of six, most were young males.

There was a large range in age and most of the subjects were male.

The SO_2 measurements were taken from only one fixed monitor in Boston, yet the subjects were from different areas of eastern Massachusetts. No information was given on the proximity of the monitoring station to any of the subjects.

Statistical significance of results was not given.

Limited data on SO_2 given because of weak association.

Odds ratios were very low.

There were no specific conclusions given regarding SO_2.

The authors outline the inclusion criteria for the study and list the restrictions utilized for subject selection. No mention was made as to whether subjects were made aware of any risks associated with the study.

This experiment only had one station measuring SO_2 concentrations. Misclassification of air pollution exposure is a potential source of bias in this study.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Saldiva, P.H.N., Lichtenfels, A.J.F.C., Paiva, P.S.O., Barone, I.A., Martins, M.A., Massad, E., Pereira, J.C.R., Xavier, V.P., Singer, J.M., Bohm, G.M.	**Internal ID:** 442

Title: Association between Air Pollution and Mortality Due to Respiratory Disease in Children in Sao Paulo, Brazil: A Preliminary Report

Year: 1994

Abstract: This work presents the results of a time series study relating air pollution and respiratory mortality in children under 5 years of age in the metropolitan area of Sao Paulo, Brazil. Daily records of mortality (excluding neonatal mortality) for the period May 1990 to April 1991 were collected along with daily records of relative humidity, temperature, SO_2, CO, particulates (PM_{10}), O_3, and NO_x concentrations. Using multiple regression methods we demonstrated a significant association between mortality due to respiratory diseases and the NO_x levels. After controlling for weather and season effects, the odds of dying due to respiratory diseases, considering the mean levels of NO_x in Sao Paulo, was estimated at 1.3 (+/- 0.13). This result is in accord with previous animal studies conducted by our group and indicates that air pollution in Sao Paulo has reached levels high enough to have adverse health effects on the exposed population.

Objective: This work represents the results of a time series study relating air pollution and respiratory mortality for children under 5 years of age in Sao Paulo, in the period 1990 – 1991.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
17.10 ± 10.13 $\mu g/m^3$	May 1990-April 1991	Male and female	Under 5 yrs	3.04±2.11 resp. deaths/day 5.41±2.44 nonresp. Deaths/day	Not reported	n/a	n/a

Observations:

There was no significant association between SO_2 and mortality.

When the regression model was applied using non-respiratory death as the dependent variable, no significant association with any pollutant was found. This finding suggests that if any association between overall child mortality and air pollution does exist, it is dependent mostly on respiratory deaths.

*Statistically significant

Author's Conclusions:

There were no specific conclusions given for SO_2 exposure and child mortality. The conclusions focused on NO_x.

"The results of this study indicate that mortality of children due to respiratory diseases in Sao Paulo was positively associated with the levels of NO_x. This association was not previously reported; most of the previous studies relating mortality to pollution described a positive association with particulates and SO_2. NO_x is present in the emissions of diesel, ethanol, and gasoline engines, whereas SO_2 is not detected in ethanol-fueled cars. The association between NO_x and SO_2 suggests that fossil fuels are mainly responsible for the deterioration of the air quality in Sao Paulo, since both gases are present in gasoline and diesel

emissions.”

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(Information on the total number of deaths not given) Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (Not enough information)Are the subjects a representative sample of the population to which extrapolations will be made? (Resp. or Nonresp. Deaths grouped according to ICD code)Were identical ‘criteria for exclusion’ applied to both cases and controls? (n/a – no recruitment) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (-) Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (No information) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Was a precise definition of what constitutes ‘exposure’ outlined before commencement of the data retrieval? (+) (Nonresp. deaths considered controls) Were appropriate controls recruited? (n/a)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (n/a – time series study)Was the timing and length of follow-up appropriate? (n/a) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (Not enough information)Were accepted assessment methods employed? (Not enough information)Were assessment measures sufficiently sensitive? (n/a)Was the nature and severity of symptoms graded against an explicitly defined scale? (No information provided) Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (-)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?

G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (+)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The association between SO_2 exposure in children and respiratory mortality was not significant in this study. However, the lack of findings may be due to many reasons including the location of the monitoring stations.

After controlling for weather and season effects, the odds of dying due to respiratory diseases in Sao Paulo was estimated at 1.3. This study provides evidence that NO_x increases susceptibility to respiratory infections and thus may conceivably increase mortality due to respiratory diseases in the exposed population. Although this study only describes association in terms of NO_x, synergic interactions should always be considered and possible interplay among pollutants causing respiratory damage is very difficult to exclude.

Confidence index:

The study was a time series study, so it was difficult to account for confounders and provide individual information.

Some confounders were considered, temperature, relative humidity and season, but the effects of these confounders were only reported for NO_x.

The monitoring stations were all from the central area of the city and may not be representative for surrounding areas. There was no indication as to what areas of the city the children were living. Within area exposure variation may be significant.

There is no information on the reliability of the monitoring data. The range of standard deviations for the eight SO_2 monitoring stations were reported (4.29-26.89) but the range of concentrations for each station was not reported.

The association between SO_2 and respiratory mortality was not significant.

There is no information on the total number of deaths.

The report is a bit confusing to follow as some of the results are reported in the Data and Methods section.

References missing in the Discussion section. The Discussion section is limited to NO_x.

This study was limited to children under the age of five. As mentioned above, this study only describes association of air pollution to mortality in terms of NO_x. Further studies should be done on synergic interactions. Data from multiple regression analysis was somewhat skewed suggesting that Poisson distribution should be considered. Furthermore, confidence intervals were not included.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Kinney, P.L., Ozkaynak, H.	**Internal ID:** 443

Title: Associations of Daily Morthality and Air Pollution in Los Angeles County

Year: 1991

Abstract: We report results of a multiple regression analysis examining associations between aggregate daily mortality counts and environmental variables in Los Angeles County, California for the period 1970 to 1979. Mortality variable included total deaths not due to accidents and violence (M), deaths due to cardiovascular causes (CV), and deaths due to respiratory causes (Resp). The environmental variables included five pollutants averaged over Los Angeles County--total oxidants (Ox), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), carbon monoxide (CO), and KM (a measure of particulate optical reflectance). Also included were three metereological variables measured at the Los Angeles International Airport--temperature (Temp), relative humidity (RH), and extinction coefficient (B_{ext}), the latter estimated from noontime visual range. To reduce the possibility of spurious correlations arising from the shared seasonal cycles of mortality and environmental variables, seasonal cycles were removed from the data by applying a high-pass filter. Cross-correlation functions were examined to determine the lag structure of the data prior to specifying and fitting the multiple regression models relating mortality and the environmental variables. The results demonstrated significant associations of M (or CV) with Ox at lag 1, temperature, and NO_2, CO, or KM. Each of the latter three variables were strongly associated with daily mortality but also were highly correlated with one another in the high-frequency band, making it impossible to uniquely estimate their separate relationships to mortality. The results of this study show that small but significant associations exist in Los Angeles County between daily mortality and three separate environmental factors: temperature, primary motor vehicle-related pollutants (e.g., CO, KM, NO_2), and photochemical oxidants.

Objective: (The objective is not clear)
We report the results of a new analysis of daily death counts and levels of environmental variables in Los Angeles County, CA during the period 1970-1979.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
15±6 ppb (average over study period)	1970-1979 (data collected)	Male and female	No info	Total daily deaths = 152	n/a	n/a	n/a

Observations:

A statistically significant association between changes in SO_2 concentrations and mortality was not observed in this study.
Pollution levels either remained steady or tended downward over this same period. The pollutant with the biggest percentage drop was SO2, which fell about 50% over the 10-year period.
NO_2, CO, and KM were strongly associated with daily mortality but also were highly correlated with one another in the high-frequency band, making it impossible to uniquely estimate their separate relationships to mortality.

Author's Conclusions:

There were no specific conclusions related to SO_2 exposure and mortality. SO_2 was not even mentioned in the conclusion.
"The results of this study show that small but significant associations exist in Los Angeles County between daily mortality and three environmental factors: temperature, primary motor vehicle-related pollutants and photochemical oxidants."
"We tentatively conclude that there is a reasonable likelihood that the results we have obtained do represent causal relationships among the variables analyzed. It

is noteworthy that in every large mortality series examined thus far, statistically significant associations of pollution and temperature with mortality have been demonstrated. This consistency across different locations and investigators lends further support to the notion that day to day fluctuations in both pollution and temperature have a real impact on daily mortality."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(n/a – reported as number of deaths/day) Is the number of subjects sufficient to produce statistically significant results? (-) study just looking at mortality ratesAre characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (no info) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a)Were identical 'criteria for exclusion' applied to both cases and controls? (n/a – time series study, no recruitment) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (+) – 10 year study period Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (No information)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a – time series study)Were appropriate controls recruited? (n/a)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (n/a)Was the timing and length of follow-up appropriate? (n/a)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a – no controls)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) determined by ICD codes Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? Not providedDid qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?

F. Data analysis:	(+)Are the statistical methods used appropriate? (-)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+/-)SO_2 not discussed in discussion and conclusion and only limited info in results Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (-)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a – no specific conclusions for SO_2) Do the conclusions make biological sense? (n/a – no extrapolations made for SO_2) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (No)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There was no significant association reported between SO_2 and total mortality. Details regarding SO_2 concentrations and respiratory mortality and cardiovascular mortality were not provided in the paper. The discussion and conclusions of the study focus on CO, O_3 and NO_x. Authors have demonstrated associations between short-term variations in total mortality and pollution, controlling for temperature.

This study is not entirely novel in that a series of studies addressing the relationship between daily mortality and pollution levels in Los Angeles began in the late 1950s. Future studies should be directed at providing insight into the mechanisms underlying possible causal relationships between pollution exposures and mortality.

Confidence index:

Extensive statistical analysis with a good explanation of the statistical methods used and references for justification.

The monitoring stations covered the whole area.

Confounders such as seasonality, temperature and relative humidity were controlled.

There were only 8 deaths/day from respiratory causes (while 87deaths/day were cardiovascular) which may have limited the ability to detect small associations. Also, deaths associated with pollution exposure would not necessarily be classified as respiratory.

To eliminate the possibility of confounding resulting from the shared seasonal cycles of mortality and environmental variables, a filter designed to remove seasonal cycles was applied to the data prior to model fitting. Filtering was performed on 10 variables, allowing substantial reduction in the variance contributed by annual and other long-period cycles. This may have reduced the statistical power.

The main weaknesses in this study are those that are seen in many case-control studies—exposure assessment and misclassifications. No conclusions were made about SO_2 and its associations with mortality.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Hoek, G., Brunekreef, B.	**Internal ID:** 444
Title: Effects of Low-Level Winter Air Pollution Concentrations on Respiratory Health of Dutch Children	
Year: 1994	
Abstract: During three consecutive winters acute effects of air pollution on respiratory health of more than 1000 children living in four nonindustrial communities in the Netherlands have been investigated. Each child performed between 6 and 10 pulmonary function tests on predetermined days. The occurrence of acute respiratory symptoms in the children was registered in a daily diary filled out by the parents of the children for periods of 3 months. Exposure to air pollution was characterized by the ambient concentration of sulfur dioxide, nitrogen dioxide, PM_{10}, fine particle sulfate, fine particle nitrate, and nitrous acid. No major winter air pollution episode occurred during these winters. Concentrations of acid aerosol were low. A weak negative association between the concentrations of nitrogen dioxide, PM_{10}, aerosol sulfate, aerosol nitrate, nitrous acid, and pulmonary function was found. Sulfur dioxide concentration was not associated with pulmonary function. No association of the concentration of the measured pollutants with daily incidence and prevalence of acute respiratory symptoms was found.	
Objective: The purpose of the present epidemiological study was to evaluate the acute effects of ambient air pollution episodes on pulmonary function and acute respiratory symptoms of children living in the Netherlands.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean SO_2 concentration on day before pulmonary function tests (14.9 ± 14.5 µg/m^3); Range 0.4-94.3 µg/m^3	Measurements taken over 3 consecutive winters	546 boys 532 girls	7-11 years	Winter 1-N=308 Winter 2-N=381 Winter 3-N=390	Predominantly healthy	n/a	n/a

Observations:

Significant positive associations between daily symptom prevalence and air pollution concentrations were found for same-day SO_2 with cough (odds ratio, 1.10) and previous-day SO_2 with lower respiratory symptoms (odds ratio 1.18).
The association of incidence of acute respiratory symptoms and SO_2 concentrations on the same and previous days was not significant.
Sulfur dioxide concentration was not associated with pulmonary function.
Before temperature adjustment, there was a slight negative association of pulmonary function with NO_2, HONO, and the aerosol components, but not with SO_2.

Author's Conclusions:

"Sulfur dioxide concentration was not associated with pulmonary function."
"No association of the concentration of the measured pollutants with daily incidence and prevalence of acute respiratory symptoms was found."
"Despite the low concentrations, a small negative association was observed between pulmonary function and the concentration of NO_2, HONO, PM_{10}, sulfate, and nitrate, but not SO_2. Adjustment for ambient temperature decreased both magnitude and significance of the mean regression slopes, but not for PM_{10} and NO_2. Daily prevalence and incidence of acute respiratory symptoms were not clearly associated with any of the air pollutants considered in this study."
"For the diary used in this study several indirect comparisons have been made. The presence of chronic respiratory symptoms according to the questionnaire significantly predicted the reporting of at least one acute respiratory symptom in the study period. The closest association was found for lower respiratory symptoms."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-)Information collected but not reported in studyAre characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (-) (No controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (+)Is the exposure duration known?
C. Equipment:	(+)Was a full description of the monitoring devices provided? (-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (-)Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (-)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (-)Were assessment measures sufficiently sensitive?

	(-)Was the nature and severity of symptoms graded against an explicitly defined scale? **(Not stated)** Did qualified personnel conduct the exams? (-)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (+)Are the methods reproducible? **(+)-except additional chemical exposure**Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? **(n/a)no extrapolations made**Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Significant positive associations between daily symptom prevalence and air pollution concentrations were found for same-day SO_2 with cough and previous-day SO_2 with lower respiratory symptoms; however the odds ratios for both symptoms were close to one. The association of incidence of acute respiratory symptoms and SO_2 concentrations on the same and previous days was not significant. Sulfur dioxide was also not associated with pulmonary function. The children in the study were predominantly healthy and the low concentrations of air pollution may have been insufficient to cause health effects. There were significant discrepancies between the symptoms reported by the parents in the diary and the symptoms reported by the children, which may have affected the results.

A small negative association was observed between pulmonary function and the concentration of NO_2, HONO, PM_{10}, sulfate, and nitrate, but not SO_2. Adjustment for ambient temperature decreased both magnitude and significance of the mean regression slopes, but not for PM_{10} and NO_2. Daily prevalence and incidence of acute respiratory symptoms were not clearly associated with any of the air pollutants considered in this study.

Confidence index:
The description of exposure assessment and methods was very detailed.
Justification was provided for assessment techniques and methods.
Confounding variables were considered, but additional exposures were not.
Baseline health information not provided.
All of the schools chosen were in nonindustrial communities and predominantly healthy. The SO_2 concentrations were measured from the closest background station. The concentrations may have been too low to cause measurable health effects.
The frequency and timing of assessment differed for each group of children. Only 6-10 pulmonary measurements taken per child each winter and testing limited to school hours.
At the time of a pulmonary function test, the children were asked whether they had experienced respiratory symptoms ("cold," cough, runny nose, throat ache, hoarseness) in the previous week. Recall bias could be an issue here and the children's answers may have been influenced by the fact that they were about to undergo pulmonary tests.
Some of the SO_2 concentrations were missing and had to be estimated or replaced using data from nearby rural areas.
The parents were responsible for filling out diaries and returning every two weeks. There was a large discrepancy between symptoms reported by parents and symptoms reported by children. A comparison of symptoms reported by the child during the pulmonary function test and symptoms reported by the parents showed considerable larger weekly prevalence reported by the children. Therefore the calculations of prevalence and incidence may be unreliable.
The presence of chronic respiratory symptoms according to the questionnaire significantly predicted diary reporting of at least one respiratory symptom in the child's study periods.
The odds ratios were very low.
Only children whose parents completed the diaries were included in the study. In two areas, the parents of more than 70% of the children completed all 2 wk diary forms. In two other areas, the parents of only 46% of the children completed all forms. This may indicate an important difference in the two groups of subjects.
In this study, the authors concede that the accuracy of diaries is questionable, considering the personal biases involved. This may have contributed to the lack of significant findings in this study. Furthermore, the communities surveyed were both industrial and non-industrial, indicating extreme variance in demographics.
Confidence intervals are not reported nor are raw and individual data. The study design does not fully limit confounding variables.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Queiros, M., Bonito-Vitor, A., Costa-Pereira, A., Costa Maia, J. | **Internal ID:** 445

Title: Childhood Asthma and Outdoor Air Pollution in Oporto Area

Year: 1990

Abstract: The influence of outdoor air pollution, on childhood asthma, is not yet completely understood, especially a long exposition to low but persistent pollutants levels. This paper presents the relationship between air pollution, as sulphur dioxide (SO_2) and black smoke (BS), and asthmatic attacks on children living in the Oporto area, during the period between 1983 and 1987, when its levels ranged below the official "security" ones. There was no correlation between daily levels of SO_2 or BS, and the asthmatic attacks rate. However, for longer periods, as months and quarters, an increased positive correlation was found, but only with SO_2 ($r = 0.334$, $p = 0.01$; $r = 0.473$, $p = 0.07$, for month and quarter periods, respectively). These data suggest that, neither SO_2 nor BS seem to be direct bronchospasm inductors, at least when its levels stay between the relatively low limits observed. On the other hand, the longer exposition to SO_2 appears to lower the threshold of the asthmatic children to other bronchospasm stimulus.

Objective: We proposed to study the influence of outdoor air pollution in childhood asthma in our area.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean 1983-1987 $= 23.9 \pm 8.1$ μg/m^3	Approximate 4 year study period	Male and female	Less than 10 years	Target Population: Approximately 100,000 children living in study area 921 emergency room visits due to asthmatic attacks 2183 asthmatic admissions to pediatric clinic	No info	n/a	n/a

Observations:

For periods of months and quarters, there is a positive correlation between SO_2 and asthmatic admissions with statistical significance, $p=0.01$ and $p=0.07$ respectively for monthly means and quarterly means

There was no correlation between daily levels of SO_2 or BS, and the asthmatic attacks rate.

For 1987, there was a significant correlation between ER visits by asthma and the corresponding admissions on the same day; it may be noticed that admissions are not simultaneously counted as ER visits.

SO_2 and BS levels, both the annual means and daily maximum levels, are found lower than the maximal recommended levels by the national and EEC legislation, and logically lower than the ones to which an increase in mortality have been associated.

BS had a much lower correlation in any situation, even when the influence of SO2 was discounted.

Author's Conclusions:

"These data suggest that neither SO_2 nor black smoke seem to be direct bronchospasm inductors, at least when its levels stay between the relatively low limits observed. On the other hand, the longer exposition to SO_2 appears to lower the threshold of the asthmatic children to other bronchospasm stimulus."

"The fact of not having found, in our work, a relation between the daily levels of SO_2 or BS and the asthmatic attack frequency, suggests that these do not act as direct inductors of the bronchospasm, maybe because the levels verified are relatively low, whether in the annual averages or whether in maximal daily values obtained…in longer periods where we are able to observe situations and effects of more prolonged exposition, there is a positive correlation between SO_2 and asthmatic admissions. So periods with higher levels of SO_2 appear to predispose to more bronchospasm episodes.

Although BS, in our study, does not seem to be an important factor in the etiology of asthma, this does not mean that they are not potential interveners. We know that they facilitate to SO_2, the access to the lower respiratory tract by absorption on its surface."

Review – Assessment: |NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.|

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (Not enough info provided)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a – no controls)Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(-) only a 4 year average givenWas the exposure concentration measured? (-)Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (-)Were appropriate controls recruited? (n/a no blinding)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+) hospital recordsWas the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a) no controls Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (Not enough info provided)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (Not enough info provided)Were accepted assessment methods employed?

	(Not enough info provided)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (No info)Did qualified personnel conduct the exams? (No info)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-) Limited infoAre the statistical methods used appropriate? (+)Are confidence intervals reported? (Not enough info)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (-)Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (+) Possibly, the primary authors work in the Pediatric Dept. of the Hospital where asthma admissions data were collected.)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were few statistical findings in this study. The authors did find that over longer periods of time there were very small significant correlations between SO_2 and asthmatic admissions. The authors claim that their findings suggest that SO_2 does not directly induce bronchospasm; however, the study lacks sufficient detail to make this claim and does not confirm its findings with other studies.

This study noted that children have some advantages as study models, in that they are generally not subjected to professional exposure or tobacco smoke in an active way.

Confidence index:

The introduction and literature review are not adequate.

The material and methods section was confusing (as far as how certain averages were calculated) and lacked sufficient detail. There was no justification for some of the methods used. Here is an example: the seasonality of the asthma attacks was calculated as follows: "the daily mean for each month was divided by the daily mean of the same year, and then the average of the five means of each month was found". The description of the statistical analysis was very limited.
The discussion section lacked sufficient detail.
There are limited data given on SO_2 concentrations.
Confounders are not considered.
The paper discusses asthmatic attacks (visits to emergency) and asthmatic admissions (pediatric clinic), but it is not always clear if they two groups are being treated separately or grouped together.
There is no information on the children (gender, age). Demographic data were not provided, nor was the range of age or the patients who visited the pediatric department. Previous health history of the subjects may have also confounded findings from this study.
This paper has been translated to English, and some of the explanations are a little hard to follow.
Confidence intervals are not reported.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Hiltermann, T.J.N., Stolk, J., van der Zee, S.C., Brunekreef, B., de Bruijne, C.R., Fischer, P.H., Ameling, C.B., Sterk, P.J., Hiemstra, P.S., van Bree, L.	**Internal ID:** 446
Title: Asthma severity and susceptibility to air pollution	
Year: 1998	

Abstract: Exacerbations of asthma have been associated with exposure to ozone or particles with a 50% cut-off aerodynamic diameter of 10 microm (PM10). We postulated in this study that the association of summertime air pollution (i.e. ozone and PM10) with acute respiratory symptoms, medication use and peak expiratory flow differs among patients grouped according to asthma severity. During the summer of 1995, effects of ambient air pollution on these parameters were studied in a panel of 60 nonsmoking patients with intermittent to severe persistent asthma. These patients were recruited from our Pulmonary Out-patient Clinic. Subgroup analysis was performed on the degree of hyperresponsiveness and lung steroid use before the start of the study, as indictors for the severity of asthma. Associations of the parameters studied with ozone, PM10, nitrogen dioxide (NO2), sulphur dioxide (SO2) and black smoke were evaluated using time series analysis. Several episodes with increased summertime air pollution occurred during the 96 day study period. Eight hour average ozone concentrations exceeded the World Health Organization (WHO) Air Quality Guidelines (120 microg x m(-3)) on 16 occasions. Daily mean levels of PM10 were moderately elevated (range 16-98 microg x m(-3)). Levels of the other measured pollutants were low. There was a consistent, positive association of the prevalence of shortness of breath (maximal relative risk (RRmax) 1.18) with ozone, PM10, black smoke and NO2. In addition, bronchodilator use was associated with both ozone and PM10 levels (RRmax 1.16). Stratification by airway hyperresponsiveness and steroid use did not affect the magnitude of the observed associations. No associations with peak expiratory flow measurements were found. We conclude that the severity of asthma is not an indicator for the sensitivity to air pollution.

Objective: In this study we hypothesized that, in patients with intermittent to severe persistent asthma, the severity of asthma (as indicated both by the amount of steroid use and airway hyper responsiveness) is the major predictor of symptoms of asthma on smog days during summer.

Overall study design:

Exposure level	Exp freq	EL	LOAEL

Observations:

SO_2 analysis is not included in this study due to low concentrations. "The SO_2 levels were not included in the analysis, since they were negligible during the study period ($<17 \mu g/m^3$)".

*Statistically significant

Author's Conclusions:

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	✎ Is the number of subjects sufficient to produce statistically significant results? ✎ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ✎ Are the subjects a representative sample of the population to which extrapolations will be made? ✎ Were identical 'criteria for exclusion' applied to both cases and controls? ✎ Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	✎ W ✎ Is
C. Equipment:	✎ W ✎ W
D. Procedural:	✎ W ? ✎ W ✎ W ✎ W ✎ W
E. Data collection:	✎ W ✎ Were appropriate endpoints examined? ✎ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) ✎ Were accepted assessment methods employed? ✎ Were assessment measures sufficiently sensitive? ✎ Was the nature and severity of symptoms graded against an explicitly defined scale? ✎ Did qualified personnel conduct the exams? ✎ Was the timing and frequency of assessment logical? ✎ Was raw data provided to permit the reader to arrive at his/her own conclusions?

SO_2 analysis is not included in this study due to low concentrations. "The SO_2 levels were not included in the analysis, since they were negligible during the study period ($<17\mu g/m^3$)".

	✓ Was individual data reported for each subject? ✓ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	✓ Are the statistical methods used appropriate? ✓ Are confidence intervals reported? ✓ Did the investigator appropriately interpret the statistics? ✓ Are the observed trends statistically significant?
G. Interpretations:	✓ Was the original objective addressed? ✓ Are the results novel? ✓ Was the design of the study appropriate to investigate the primary objective? ✓ Were assumptions reasonable? ✓ Are the methods reproducible? ✓ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? ✓ Do the conclusions make biological sense? ✓ Are the extrapolations reasonable? ✓ Is the paper in a peer-reviewed journal? ✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:
Reviewer's summary of study findings and interpretation of the toxicological significance and clinical relevance of these findings.
Confidence index:
Reviewer's assessment of the quality of the study design, conduct and reporting.

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM

Author: Hong, Z.	**Internal ID:** 447

Title: Serum Clara Cell Protein (CC16): A New Valid Marker of the Distal Airway Damages Caused by Air Pollutants in Rats

Year: 1996

Abstract: Cell count, LDH, total protein, Clara cell protein (CC16) and lysozyme in bronchoalveolar lavage fluid (BAL) and serum were determined in rats. 30-50 ppm sulfur dioxide for 4h, 12h, and 2 week exposure didn't produce any change of above parameters. 1.0ppm, 0.5ppm, 0.38ppm, 0.25ppm and 0.20ppm ozone for 7 h exposure tests were performed. CC16, total protein and LDH in BAL had significantly changes in BAL in ozone groups. Only serum CC16 showed significant increase along ozone groups. Both BAL and serum CC16 were of the best exposure response relationship with ozone concentration. The change of BAL fluid reached high peak 18-24 h after ozone exposure. Serum CC16 seemed to be a valid marker of the distal airway damages caused by air pollutants.

Objective: The study investigated the possibility of using a serum CC16 as a potential marker reflecting the lung tissue damage caused by air pollutants (sulfur dioxide and ozone) and explored the toxicological mechanism of above air pollutants.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
Six Experiments: 1: 30ppm 2,3,4: 50ppm 5: 50ppm 6: 50ppm	Six Experiments: 1. 4h 2. 4h (repeated 3x) 3. 12h 4. 2 weeks	Rats	Sprague Dawley	3-6 months	female	N=80 Six Experiment(includes controls) s: 1. n=10 2,3,4. n=30 5. n=20 6. 20	Specific pathogen free		

Observations:

Changes of Bronchoalveolar Lavage Fluid (BAL)

There was no obvious change of cell count, LDH, total protein, CC16 and lysozyme in BAL among all exposure groups compared to the controls.

Effect on Serum

Non-significant change was observed in total protein, LDH, CC16 and lysozyme in the serums among the SO_2expsoed groups compared with controls.

Author's Conclusions:

> "Sulfur dioxide is a highly water soluble gas, in contrast to the common air pollutant ozone. Therefore, up to 98% may be absorbed in the nasopharynx during nasal breathing. Consequently, according to the current concept, only a minor portion will reach the proximal pulmonary airway and, due to the high water solubility, the rest will be rapidly absorbed in the mucous and epithelial lining fluid in the bronchi. Little or none of the SO_2 is believed to reach the lower airways. Therefore, no significant change in BAL and serum parameters was observed in rats exposed to 30-50 ppm during 4h to 2 weeks. These results are in line with William's observation which also showed no significant reduction in macrophage endocytosis in resting hamsters exposed for 4h to 50ppm SO_2."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	**(+) but female only** Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* **(-) 5 months)** Age *Guideline: Adult, i.e.,8-12 weeks.* **(+) female)** Sex (+) d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* **(-) greater than 20%** e) Weight variation *Guideline: Below 20% variation* **(no info)** f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	**(+) except lot** Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? **(-) no justification given for levels** .Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(no info)** Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary

	objective? **(+)** Was the duration of exposure precisely defined? **(no info)** If exposure chambers were employed, were animals exposed during the equilibration period? ↳ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(+)** Was the mode of administration appropriate? **(+)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? **(not stated)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(no details)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? ↳ *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **((+) in some cases 10 animals** Was the number of animals grouped in a single chamber stated? ↳ *Guideline: Groupings should permit clear observation of each animal.* **(+)** Was the manner by which animals were grouped appropriate? ↳ *Guideline: Animals may be grouped by sex, or individually.* **(-)** Was the type and source of feed and water stated? Was it appropriate? ↳ *Guideline: Conventional lab diet* **(+)** Was the amount and feeding schedule specified? ↳ *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(+) just dimensions** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. ↳ *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(-)** Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?

E. Procedural:	**(not stated)** Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* **(just diet)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(not stated)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? (+) Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (-) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (-) Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective?

	(more detail needed) Were assumptions reasonable? **(-)** Are the methods reproducible? **(more detail needed)** Did either the study design or the statistical analysis limit confounding variables? **(n/a)** Do the conclusions make biological sense? **(no extrapolations made for SO_2)** Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There was no significant change in bronchoalveolar levels and serum parameters were observed in rats in this study. There were no statistically significant findings for SO_2. This may have been due to the insensitivity of the assessment techniques. Or, as stated by the authors, the lack of significant results may be an indication of the fact that SO_2 does not reach the lower airways.

Confidence index:

The weight variation was greater than 20%.
There was no justification given for the exposure levels used.
There is no mention of random assignment.
There were no details on the climate or acclimation conditions of the chamber.
The methods section was very brief.
There was limited information on the monitoring devices.
The authors attempted to limit confounding factors by listing age, species, health and weight of the rats.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Segala, C., Fauroux, B., Just, J., Pascual, L., Grimfeld, A., Neukirch, F.	**Internal ID:** 448

Title: Short-term effect of winter air pollution on respiratory health of asthmatic children in Paris

Year: 1998

Abstract: There is controversy as to whether low levels of air pollution affect the symptoms and lung function in asthma. We addressed this by examining the short-term effects of winter air pollution on childhood asthma in Paris. We performed a 6-month follow-up of 84 medically diagnosed asthmatic children classified into two groups of severity. The outcomes included incidence and prevalence of asthma attacks, symptoms and use of supplementary β-2-agonists, peak expiratory flow (PEF) value and its variability. The statistical analysis controlled the lack of independence between daily health outcomes, trends and meteorology. Air pollution was associated with an increase in reports and duration of asthma attacks and asthma-like symptoms in mild asthmatic children. The strongest association was the risk of asthma attack for an increase of 50 μg x m^{-3} of sulphur dioxide (SO_2) on the same day (odds ratio (OR)=2.86). Maximum reduction in morning peak expiratory flow (PEF) (5%) and maximum increase in PEF variability (2%) were observed at a lag of 3 days for an increase of 50 μg x m^{-3} of SO_2 in the subgroup of mild asthmatics receiving no regular inhaled medication. In moderate asthmatic children, the duration of supplementary β-2-agonist use was strongly associated with air pollution. The general pattern of our results provides evidence of the effect of the low levels of air pollution encountered in Western Europe on symptoms and lung function in childhood asthma.

Objective: The purpose of the present study was to examine the short-term effects of winter air pollution on the respiratory health of medically diagnosed asthmatic children. We used a panel study, controlling for the lack of independence of daily health outcomes and considering both maintenance and supplementary medications taken by the subjects.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
11 stations **Mean – 21.7±13.5 μg/m^3** **Range – 4.4-83.8 μg/m^3**	**25 weeks**	**53 boys** **31 girls**	**Mild asthmatics – 9.6±2.3y** **Moderate e asthmatics- 10.6±2.5y**	**Mild asthmatics -43** **Moderate asthmatics -41**	**Medically diagnosed asthma**	**n/a**	**n/a**

Observations:

The risk for an increase in incidence of asthma attack in mild asthmatics with an increase of 50μg/m^3 of SO_2 was significant for the same day (OR=2.86 (1.31-6.27)) and for lag 1 day (OR = 2.45 (1.01-5.92)).

The risk for an increase in incidence of nocturnal cough in mild asthmatics with an increase of $50\mu g/m^3$ of SO_2 was significant for the lag 3 days (OR=1.93 (1.18-3.11)) and for lag 4 days (OR = 2.12 (1.43-3.13)).

The risk for an increase in incidence of respiratory infections in mild asthmatics with an increase of $50\mu g/m^3$ of SO_2 was significant for the lag 3 days (OR=2.39 (0.9-6.37))

The risk for an increase in incidence of supplementary β_2-agonist use in mild asthmatics with an increase of $50\mu g/m^3$ of SO_2 was significant for the lag 4 days (OR=1.63 (1-2.66)) .

The risk for an increase in prevalence of asthma attacks in mild asthmatics with an increase of $50\mu g/m^3$ of SO_2 was significant for the lag 0 day – OR=1.71 (1.15-2.53).

The risk for an increase in prevalence of wheeze in mild asthmatics with an increase of $50\mu g/m^3$ of SO_2 was significant for the lag 4 days – OR=1.48 (0.90-2.41).

The risk for an increase in prevalence of shortness of breath in mild asthmatics with an increase of $50\mu g/m^3$ of SO_2 was significant for the lag 2 days (OR=1.45 (0.98-2.14)) and for lag 3 days (OR = 1.52 (1.03-2.25)) and lag 4 days (OR=1.51 (1.02-2.24)).

The risk for an increase in prevalence of respiratory infection in mild asthmatics with an increase of $50\mu g/m^3$ of SO_2 was significant for the lag 3 days (OR=2.09 (1.05-4.15)) and for lag 4 days (OR = 2.05 (1.14-3.68)).

The risk for an increase in prevalence of supplementary β_2-agonist use in mild asthmatics with an increase of $50\mu g/m^3$ of SO_2 was significant for the lag 4 days (OR=2.02 (1.02-4.01)) .

The risk for an increase in prevalence of supplementary β_2-agonist use in moderate asthmatics with an increase of $50\mu g/m^3$ of SO_2 was significant for the lag 0 day (OR=3.67 (1.25-10.8)), lag 1 day – OR=4.60 (2.10-10.1), lag 2 days – OR=7.01 ((3.53-13.9)) and lag 3 days – OR=4.74 (1.96-11.5). .

In a subset of the mild asthmatics (n=21) with no inhaled steroids and no regularly scheduled β_2 agonists there were some correlations between PEF variables and SO_2exposure. Daily PEF variability increased by 1.9% (not significant) for an increase of $50\mu g/m^3$ of SO_2 (maximum increase was approximately 3.8% (stat. significant) at lag 3 days.)

SO2 was associated with: both incident and prevalent episodes of asthma; use of supplementary β_2-agonists; incident episodes of nocturnal cough; prevalent episodes of shortness of breath; and respiratory infection. Symptoms were more strongly associated with lagged than concurrent-day pollutant levels, except for asthma attack for which the risk for an increase of 50 ug/m^3 of SO_2 was the highest on the same day.

Author's Conclusions:

"In conclusion, we have shown that prevailing levels of winter air pollution, which are below international air quality standards, had consistent and measurable effects on children with mild to moderate asthma. These effects lasted several days after exposure, suggesting a persistent inflammatory process. We also showed that moderate asthmatic patients could manage their bronchial responses to air pollution by treatment."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)**Were identical 'criteria for exclusion' applied to both cases and controls? (-) **More information needed** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (+) **6 month study period** Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? **(no info provided)**What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a – no controls)** Were appropriate controls recruited? (+)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)**Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale?

	(-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (+)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (-)Did the investigator appropriately interpret the statistics? (some)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (+).Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The findings suggest that moderately elevated SO_2 levels were associated in mild asthmatic children with increases in the incidence and duration of asthma attacks and asthma-like symptoms and with alterations of lung function as measured by reduction in PEF and increase in PEF variability. In moderately asthmatic children receiving daily treatment, both with inhaled steroids and inhaled β_2– agonists, only supplementary β_2– agonists use was strongly associated with air pollution. All these associations were observed at levels below the current acceptable standard air quality, in a homogenous group of 84 currently asthmatic children diagnosed by their hospital pulmonary pediatricians. The reduction in PEF value and the increase of PEF variability were only reported in the subgroup of mild asthmatic children with no inhaled steroids and no regularly scheduled β_2 – agonists, suggesting that anti inflammatory treatment decreases the bronchial response to air pollution. The weaker associations of asthma attacks and SO_2 exposure in the moderate asthmatic group may be due to a more efficient maintenance treatment and are better at managing their symptoms with supplementary medication. Though some of the associations were statistically significant, the odds ratios were low and the confidence intervals were wide (see Observation section). Some of the intervals included OR=1.

Confidence index:

Selection bias is possible due to the fact that the subjects volunteered and were not chosen randomly. It is not clear whether the subjects knew the details of the study before volunteering. The authors claim that the parents were not aware that the focus of the study was air pollution. The recruitment process was clearly described and selected only participants that fell within the age and health requirements. Reasons for exclusion were also noted.
Only children who had at least one asthma attack in the past 12 months were included. It is not stated whether this is from medical records or self reported.
There are no details on the 16 children who did not complete the study. (Original number n=100).
In the mild asthmatic group, 21 children who received no regular medications appeared to be more sensitive than the remainder of the mild group.
Children were responsible for taking PEF values and recording them. Children were also asked to record times spent outside of study area.
Parents completed diaries at the end of the day. Much of the information would have to be collected from the children who would be at school all day. May introduce some recall bias.
The medications may reduce the susceptibility to the air pollution.
The study took place in the winter, when children may be spending most time indoors.
The average of the mean daily readings at all SO_2 stations was justified by referencing a study that verified the temporal and geographical homogeneity between data collected in the various stations. Thus the subjects were considered as all having a similar exposure. However, activity levels and additional chemical exposure information was not provided.
Confounders were considered in the analysis.
Training effect was considered.
There was an attempt at blinding. The subject's parents were given a diary and instructed how to complete it. Participants were told that this was an investigation of factors related to severity of asthma but not that the principle factor of interest was air pollution.
Asthma was medically diagnosed.
Some consistency reported with other studies.
The discussion was extensive.
Exposure assessment is a weakness in this type of study. However, patients were asked to note the days spent outside the study area, and these days were excluded from the analysis. The accuracy/sensitivity of monitoring devices and raw data are not given.

Review – Confidence index ranking:

Moderate to low

CASE-CONTROL REVIEW FORM

Author: Roemer, W., Hoek, G., Brunekreef, B.	**Internal ID:** 449

Title: Effect of Ambient Winter Air Pollution on Respiratory health of Children with Chronic Respiratory Symptoms

Year: 1993

Abstract: The acute respiratory effects of ambient air pollution were studied in a panel of 73 children with chronic respiratory symptoms in the winter of 1990 to 1991. The participating children were selected from all children aged 6 to 12 yr in Wageningen and Bennekom, two small, nonindustrial towns in the east of the Netherlands. Peak flow was measured twice daily with MiniWright meters. A diary was used to register the occurrence of acute respiratory symptoms and medication use by the children. Exposure to air pollution was characterized by the ambient concentrations of sulfur dioxide (SO_2), nitrogen dioxide (NO_2), black smoke (BS), and particulate matter less than 10 microns (PM_{10}). Associations between air pollution concentrations and health outcomes were analyzed using time series analysis. During the study period an air pollution episode occurred, with moderately elevated concentrations of PM_{10} and SO_2. There were 6 days with 24-h average PM_{10} concentrations in excess of the WHO suggested lowest observed effect level of 110 $\mu g/m^3$. After adjustment for ambient temperature, there were small but statistically significant negative associations of PM_{10}, BS, and SO_2 with both morning and evening PEF. There was a consistent positive association between PM_{10}, BS, and SO_2 with the prevalence of wheeze and bronchodilator use. Overall, the observed associations suggest a mild to moderate response to these moderately elevated levels of air pollution in a group of potentially sensitive children.

Objective: …to perform a study of the acute effects of winter air pollution on respiratory health in a panel of children with chronic respiratory symptoms.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Highest observed 24 h average – 105 $\mu g/m^3$ **Highest observed 1h max – 147 $\mu g/m^3$**	**Continuous**	**33 female** **40 male**	**9.3±1.7 years**	73	**Chronic respiratory symptoms**	**n/a**	**n/a**

Observations:

After adjustment for ambient temperature, there were small but statistically significant negative associations of SO_2 with both morning and evening Peak Flow.
Several respiratory symptoms showed a weak but not statistically significant positive correlation with SO_2.
During the study period an air pollution episode occurred, with moderately elevated concentrations of PM_{10} and SO_2.
After adjustment for ambient temperature, there were small but statistically significant negative associations between PM_{10}, BS and SO_2 with the prevalence of

wheeze and bronchodilator use.
A positive association was found in the logistic model for phlegm incidence with same day SO_2 and PM_{10} and runny nose with SO_2. Confidence intervals were wide, however. Several respiratory symptoms showed a weak positive correlation with SO_2, PM_{10}, and black smoke, but not with NO_2. Wheeze showed the most consistent positive association with the various exposure indices.

Author's Conclusions:

"Moderately elevated concentration levels of PM_{10}, SO_2 and black smoke were associated with small but statistically significant decrements of peak flow in a panel of children with chronic respiratory symptoms."

"There was also a positive correlation between the prevalence of bronchodilator use and SO_2."

"In conclusion, moderately elevated concentrations of wintertime PM_{10} and SO_2 were associated with small decrements in peak flow and increases in wheeze and bronchodilator use in a panel of children with chronic respiratory symptoms."

"The observation of associations of moderately elevated concentrations of air pollution characterized by particulate matter and SO_2 with respiratory effects is consistent with several recent studies. Increased hospital admissions were associated with higher PM_{10} concentrations in the Utah valley and increased SO_2 and black smoke concentrations in Barcelona. The results of our study are nearly identical to the results recently reported from a panel study with a similar design in the Utah Valley. The magnitude of the observed associations was relatively small. The small peak flow decrements and symptom increases may have been mitigated by increased bronchodilator use. Weekly average concentrations had stronger relationships with symptoms and medication than present day or 1 day lagged concentrations."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+)Are the subjects a representative sample of the population to which extrapolations will be made? n/a – no controlsWere identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (assumed continuous)Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (no information)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval?

	(n/a)Were appropriate controls recruited? (n/a – no blinding)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (+)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (+)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (some)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? (no extrapolations made)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (No)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

After adjustment for ambient temperature, there were small but statistically significant negative associations of SO_2 with both morning and evening PEF. There was also a positive correlation between the prevalence of bronchodilator use and SO_2 that was not statistically significant. The SO_2 concentrations levels were well below the suggested WHO guideline. It is also not clear from the presented data what components of the episode mixture were responsible for the observed respiratory effects. It might be questioned whether exposure to air pollution was adequately characterized by fixed-site ambient air concentrations only, since people spend a large proportion of their time indoors. If so, the resulting misclassification of exposure would probably have resulted in a an attenuation of the observed association towards the null.

Confidence index:

Daily measurements of peak expiratory flow were made using peak flow meters and diaries used to record symptoms.
Individual data for each child studied to identify potential trends or irregularities. Data considered unreliable was eliminated from study.
Potential training effects were controlled for by eliminating the first week of data.
The statistical methods were described in extensive detail.
Both prevalence and incidence were clearly defined.
Reasons given for all withdrawals and/or data eliminated.
Good details on effects of weather conditions with references to other studies.
The discussion was very detailed with results compared with similar studies (with both consistent and no consistent results). Most studies had consistent results.
The authors address that the power of the study to use incidence data may not have been sufficient. This is supported by the lack of association between temperature and incidence.
The odds ratios were low and the confidence intervals were wide (which the author addresses).
Excellent description of methods.
Many possible confounders taken into effect.
Parents had to rate the severity of the symptoms, which may result in some bias.
Fixed monitoring sites may not adequately represent exposure. Information on monitoring devices not presented.
Faults that did exist were identified and discussed in the study.

Review – Confidence index ranking:

Moderate

CASE-CONTROL REVIEW FORM

Author: Braun-Fahrlander, C., Ackermann-Liebrich, U., Schwartz, J., Gnehm, H.P., Rutishauser, M., Wanner, H.U.	**Internal ID:** 450

Title: Air Pollution and Respiratory Symptoms in Preschool Children

Year: 1992

Abstract: A diary study on a random sample of 625 Swiss children aged 0 to 5 yr was conducted in two cities in Switzerland to investigate the association between air pollution and respiratory symptoms. Total suspended particulates (TSP), SO_2 and NO_2 were measured by city monitor. In addition, passive samplers inside and outside the home measured NO_2 concentration during the 6 wk each child was on the diary. Diaries were filled out by parents, and 20% were validated with the attending pediatrician's case notes. Incidence and duration of symptom episodes were examined separately. The study included any episode, episodes of coughing without runny nose, upper respiratory episodes, and episodes of breathing difficulty. In regressions using 6-wk average pollution that controlled for medical history, NO_2 measured outdoors but not indoors was associated with the duration of any symptom. Total suspended particulates were a more significant predictor of duration of any symptom than NO_2. The 6-wk average TSP was significantly associated with incidence of coughing episodes and marginally significant as a predictor of upper respiratory episodes. Previous day's TSP was a significant predictor of incidence of upper respiratory symptoms. Annual average of NO_2 was associated with the duration of any episode and of upper respiratory episodes. We conclude that the incidence and duration of respiratory symptom episodes are likely associated with particulate concentrations and duration may be associated with NO_2.

Objective: The principle hypothesis of this study was that moderate-term (6wk) exposure to air pollution is associated with an increased incidence and duration of respiratory symptoms in preschool children, a population with airways particularly sensitive to the irritant effects of air pollution.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
20-70 μg/m^3 (concentration range in two cities) 8-12 μg/m^3 (rural); 30-40 μg/m^3 (suburbs); 50-70 μg/m^3 (cities)	6 wk study period-assumed continuous	299 boys 326 girls	Under 2yr-n=279 2-5yr- n=346	625	Relatively healthy-Respiratory health background provided	n/a	n/a

Observations:

No significant associations were reported for SO_2. The study primarily focuses on NO_x.
SO_2 was not associated with incidence of cough in incidence models. No results were given in this study for duration models for daily means and annual means.

However, many significant results were found involving NO_2 and TSP.

Author's Conclusions:

There were no conclusions given for SO_2 exposure.

"We conclude that the incidence and duration of respiratory symptom episodes are likely associated with particulate concentrations and duration may be associated with NO_2."

"The correlation between outdoor passive NO_2 sampler and TSP measurements was quite high (0.52) and indeed was as high as the correlation between the passive NO_2 measurement and the city NO_2 monitors. Moreover, when both pollutants were simultaneously included in the model for symptom duration, TSP remained significant with no change in effect size, and NO_2 became insignificant. This argues for TSP as the responsible agent. TSP also showed a stronger trend in analyses of the duration of coughing and upper respiratory symptoms, although outdoor NO_2 correlated more strongly with breathing difficulty."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (Not enough information provided)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a-no controls)Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (+ 6wk study period) Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (no info)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a-no controls)Were appropriate controls recruited? (n/a)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (-)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale?

	(+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-)Are the statistical methods used appropriate? (-)Are confidence intervals reported? (-)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(-) not for SO_2 Was the original objective addressed? (-)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a no conclusions re SO_2). Do the conclusions make biological sense? (none made) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (+)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Sulfur dioxide showed no significant associations.

Confidence index:

The authors collected data from rural areas, suburbs, and cities, but only the data from the children living in the cities was analysed.

The discussion and conclusion sections do not mention SO_2.

There is no tabulated or graphical data for SO_2. The authors only state briefly that there were no significant associations with SO_2.

This study utilized diary data to assess the impact of short-term exposure to air pollution. The subject group was selected randomly. The author also described the recruitment process and rationale for inclusion and exclusion of participants. Demographic information was considered as a possible confounding factor, but found to be not statistically significant to the findings.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Henry, R.L., Bridgman, H.A., Wlodarczyk, J., Abramson, R., Asler, J.A., Hensley, M.J.

Internal ID: 451

Title: Asthma in the Vicinity of Power Stations: II. Outdoor Air Quality and Symptoms

Year: 1991

Abstract: To assess longitudinally the effect of living in the vicinity of coal-fired power stations on children with asthma, 99 schoolchildren with a history of wheezing in the previous 12 months were studied for 1 year, using daily diaries and measurements of air quality. The children had been identified in a cross-sectional survey of two coastal areas: Lake Munmorah (LM), within 5 km of two power stations, and Nelson Bay (NB), free from major industry. Daily air quality [sulphur dioxide (SO_2) and nitrogen oxides (NOx)], respiratory symptoms, and treatment for asthma were recorded throughout the year. Measurements of SO_2 and NOx at LM were well within recommended guidelines although they were several times higher than at NB: maximum daily levels in SO_2 (μg/m^3) were 26 at LM, 11 at NB (standard, 365); yearly average SO_2 was 2 at LM, 0.3 at NB (standard, 60); yearly average NOx (μg/m^3) was 2 at LM, 0.4 at NB (standard, 94). Marked weekly fluctuations occurred in the prevalence of cough, wheezing, and breathlessness, without any substantial differences between LM and NB. Overall, the prevalence of symptoms was low (10% for wheezing, 20% for any symptom). Whether the daily SO_2 and NOx levels affected the occurrence of respiratory symptoms was investigated in children at LM using a logistic regression (Korn and Whittemore technique). For these children as a group, air quality measurements were not associated with the occurrence of symptoms.

Objective: The aims of the study were:

1. to document the day-to-day fluctuation of symptoms of asthma in primary schoolchildren in Lake Munmorah (LM) and Nelson Bay (NB) over the 1 year period between prevalence surveys; and
2. to compare the prevalence and severity of asthma with measures of environmental air quality.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Low – 0 μg/m^3 Med-0-28.6 μg/m^3 High->28.6 μg/m^3	1 year study period	Male and female	School children	99	History of wheezing in past 12 months	n/a	n/a

Observations:

SO_2 concentrations were zero at LM (cases) for 95% of the study and NB for 99% of the study. Measurements of SO_2 and NOx at LM were well within recommended guidelines although they were several times higher than at NB; maximum daily levels in SO_2 (ug/m^3) were 26 at LM, 11 at NB; yearly average SO_2 was 2 at LM, 0.3 at NB. Marked weekly fluctuations occurred in the prevalence of cough, wheezing, and breathlessness, without any substantial differences between LM and NB. Overall, the prevalence of symptoms was low (10% for wheezing, 20% for any symptom).

No obvious correlation was apparent between SO_2 daily maximums and proportion of children wheezing or for any other symptoms.
Six children had significant odds ratios, indicating that they were more likely to have any symptoms on a day of "high" SO_2.

Author's Conclusions:

"In the present study, we have assessed whether current levels of air pollution at LM might be causing exacerbations of asthma. We found no evidence to support this: firstly, air quality measurements of SO2 and NOx at LM were well within recommended guidelines; secondly, symptoms of asthma were similar at LM and NB and the frequency of these symptoms was low; thirdly on average, air quality had no deleterious effect on asthmatic symptoms."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (Not enough info provided)Are the subjects a representative sample of the population to which extrapolations will be made? (+)Were identical 'criteria for exclusion' applied to both cases and controls? (Not enough info)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (+)Is the exposure duration known?
C. Equipment:	(+)Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (+)Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(+)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (-)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale?

	(-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (some)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (+)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (-)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? (+)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:
There was no evidence found in this study to support an association between the reported levels of SO_2 and exacerbations of asthma. The air quality measurements of SO_2 at LM were well within recommended guidelines. Symptoms of asthma were similar at LM and NB and the frequency of these symptoms were low. On average, air quality had no deleterious effect on asthmatic symptoms. There were some significant results for six children from the case area (LM) indicating the possibility of insufficient power in the study to detect sub-group associations.

Findings suggest that in study period, SO_2 and NOx levels were not causing exacerbations. There was little statistical evidence to support an effect of overall worsening of symptoms with exposure.

Confidence index:
This study was generally well designed. Most of the faults were addressed by the authors. The findings were consistent with many studies reported in the Discussion Section.

A control group was included for comparison.
Very clear statement of study objectives.
Very detailed material and methods section.
Confounders not considered.
SO_2 measurements were environmental, not personal (addressed by authors).
Some individual data were discussed, but details not given in study.
Power may not have been sufficient to detect important subgroup associations (addressed by authors).
Limited baseline data on subjects.
Children recruited by self-reporting some wheeziness in the past 12 months. Possible recall bias.
Symptom data collected from diaries completed by parents. Potential for bias.
The study subdivided exposure levels into "low", "medium" and "high" but did not give an explanation as to how each range was determined.
Individual and raw data were not reported. Sensitivity of monitoring devices was not stated. Withdrawals are listed but no reason for withdrawal is given. To minimize inconsistencies in symptom classification, nurses were assigned to check diary card data.

Review – Confidence index ranking:

Low to Moderate

NON-CLINICAL REVIEW FORM

Author: Ito, M., Kaniwa, T., Horiuchi, H., Nonaka, T., Yamanada, Y., Uno, H., Kishimot, T., Kiyoki, M.	**Internal ID:** 452
Title: Effect of Clenbuterol on Sulfur Dioxide Induced Acute Bronchitis in Guinea Pigs	
Year: 1995	
Abstract: When guinea pigs were exposed to sulfur dioxide (SO_2) gas (800 ppm, 2 h), they showed hyper responsiveness to intravenously administered serotonin (5-hydroxytryptamine (5-HT)). This hyper responsiveness continued for over 24 h after the exposure to the gas. The degeneration, desquamation of epithelium, and edema of the lamina propriety of the trachea and bronchi were observed in animals after a 2-h exposure of SO_2 histopathologically. These changes seemed to be the early phase of acute bronchitis. Then, we examined the effect of clenbuterol, a selective beta-2 adrenoceptor agonist, on the SO_2-induced bronchial hyperresponsiveness in these animals. Orally administered clenbuterol (1-10 μg/kg) suppressed the hyperresponsiveness to 5-HT in a dose-dependent manner. These results suggest that clenbuterol might inhibit the hyperresponsiveness that accompanies acute bronchitis and that this agent may be useful for remission of broncho-spasm.	
Objective: To study the effect of clenbuterol on sulfur dioxide induced acute bronchitis in guinea pigs.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
800ppm	2h	Guinea pigs	Hartley	4wks	male	Not clearly stated	No info	n/a	n/a

Observations:

Exposure to SO_2 at 800 ppm for 2hours caused an immediate increase in bronchial responsiveness to 5-HT that continued for over 24h.

From the histopathological study, acute mucosal injury was observed 2 h after the exposure to SO_2 gas.

The degeneration, desquamation of epithelium, and edema of the lamina propria of the trachea and bronchi were observed in animals after 2h exposure of SO_2 histopathologically.

The Alb concentration in BALF was markedly increased 2 h after the end of SO_2gas exposure, in the contrary, no significant change in the total cell counts in BALF was observed at this time.

The Alb concentration increased rapidly after the gas treatment but decreased gradually from 6h.

The total number of cells recovered by BAL increased time-dependently following SO_2exposure.

Oral administration of clenbuterol to guinea pigs markedly inhibited the onset of bronchial hyper responsiveness to 5-HT in a dose dependent manner.

Author's Conclusions:

> "The inhibitory effect of clenbuterol on bronchial hyperresponsiveness might be caused, in part, by anti-inflammatory effect, although broncho-dilation is probably the main mechanism. From our study we conclude that clenbuterol inhibits the hyperresponsiveness that accompanies acute bronchitis and that this agent may be useful for remission of broncho-spasm in acute bronchitis."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	**(not clearly stated)** Is the number of animals sufficient to produce statistically significant results? ! *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? **(+) no justification given** a) Specie ! *Guideline: Rat, or justification for use of another mammalian specie.* **(-) 4 weeks**) Age ! *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex **(no info)** d) Pre-test health ! *Guideline: Females should be nulliparous and non-pregnant.* **(approx. 25%)** e) Weight variation ! *Guideline: Below 20% variation* (+) f) Source ! *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	**(some)** Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (-) Was there a gradient of exposure levels? ! *Guideline: Three exposure levels in addition to control.* (-) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (no info) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined?

	(n/a no chamber) If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? ((-) Was evidence provided to justify the dosing regime? **(no info)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(no info)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(no)** Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* **(no info)** Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (+) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* (+) Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	(+) Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* (-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	(-) Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days*

	(+) diet Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(not stated)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(not stated)** Was a control group assayed? **(n/a)** Is the control regime appropriate? **(-)** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	**(+)** Were appropriate endpoints examined? **(-)** Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(no info)** Did qualified personnel conduct the exams? **(+)** Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each test animal?
G. Data analysis:	**(+)** Are the statistical methods used appropriate? **(+)** Are confidence intervals reported? **(+)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
H. Interpretations:	**(+)** Was the original objective addressed? **(-)** Are the results novel? **(-) more detail needed** Was the design of the study appropriate to investigate the primary objective? **(more detail needed)** Were assumptions reasonable? **(-)** Are the methods reproducible? **(more detail needed)** Did either the study design or the statistical analysis limit confounding variables? **(+)** Do the conclusions make biological sense?

	(-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

In this study, clenbuterol was able to inhibit the hyperresponsiveness that accompanied acute bronchitis in guinea pigs. The results suggest that clenbuterol may be useful for remission of bronchospasm in guinea pigs and further study is warranted. The concentration used in this study (800ppm) was not representative of the average human exposure and the guinea pigs were exposed through a tracheal cannula.

Findings in this study indicate that there are two different responses from direct epithelial injury caused by SO_2 inhalation resulting in loss of epithelial cells and an increase in permeability. Secondly, inflammatory cells, especially neutrophils, were observed in BALF, suggesting that the inflammatory cells may promote rather than initiate bronchial responsiveness.

Confidence index:

There was limited information on exposure conditions, housing/feeding, and equipment.

There was no information on the control regime and whether the assignment was randomized.

The methods section is fairly brief, and more detail is needed on the assessment techniques.

The objective is not clearly stated.

The guinea pigs were all male

The sample size is not clearly stated. (Under one figure, all that is mentioned is n=3 or 4).

There is some info on the monitoring devices, but not a full description.

The weight variation is approx. 25%.

The age of the mice (4weeks) was less than that recommended for studies (8-12 weeks).

There is no mention of whether animals were acclimated, or how they were randomly divided into groups.

There is no evidence to justify dosing levels. Pretest information was not reported.

Review – Confidence index ranking:

low

CASE-CONTROL REVIEW FORM

Author: Ponka, A.	**Internal ID:** 453
Title: Asthma and Low Level Air Pollution in Helsinki	
Year: 1991	
Abstract: The effects of relatively low levels of air pollution and weather conditions on the number of patients who had asthma attacks and who were admitted to a hospital were studied in Helsinki during a 3-y period. The number of admissions increased during cold weather (n = 4,209), especially among persons who were of working age but not among children. Even after standardization for temperature, all admissions, including emergency ward admissions, were significantly correlated with ambient air concentrations of nitrogen dioxide (NO_2), nitric oxide (NO), sulfur dioxide (SO_2), carbon monoxide (CO), ozone (O_3), and total suspended particulates (TSP). Regression analysis revealed that NO and O_3 were most strongly associated with asthma problems. Effects of air pollutants and cold were maximal if they occurred on the same day, except for O_3, which had a more pronounced effect after a 1-d lag. The associations between pollutants, low temperature, and admissions were most significant among adults of working age, followed by the elderly. Among children, only O_3 and NO were significantly correlated with admissions. Levels of pollutants were fairly low, the long-term mean being 19.2 $\mu g/m^3$ for SO_2, 38.6 $\mu g/m^3$ for NO_2, 22.0 $\mu g/m^3$ or O_3, and 1.3 mg/m^3 for CO. In contrast, the mean concentration of TSP was high (76.3 $\mu g/m^3$), and the mean temperature was low (+ 4.7 degrees C). These results suggest that concentrations of pollutants lower than those given as guidelines in many countries may increase the incidence of asthma attacks.	
Objective: In Helsinki, where there is a population of 0.5 million, we investigated the incidence of asthma attacks that required treatment in hospital wards. This incidence was correlated with ambient air SO_2, NO, NO_2, CO, O_3, TSP concentrations, temperature, relative humidity and wind speed.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean -19.2 $\mu g/mg^3$ ±12.6 Range-0.2-94.6	3 year study period	Male and female	0-14y-1359 cases 15-64y-1685 cases >64y-1165 cases	4209 hospitalizations for asthma over 3 year study period	No info	n/a	n/a

Observations:

Levels of pollutants were fairly low, the long-term mean being 19.2 $\mu g/m^3$ for SO_2, 38.6 ug/m^3 for NO_2, 22.0 $\mu g/m^3$ for O_3, and 1.3 mg/m^3 for CO.

Highly significant negative correlations were obtained when SO_2, NO_2, and NO were compared with temperature, whereas O_3 correlated positively with temperature.

The frequency of all admissions for asthma was highly significantly correlated with daily concentrations of SO_2, nitrogen dioxide, nitric oxide, carbon monoxide,

ozone, and total suspended particulates and with the minimum hourly temperature.
After standardization for temperature, the number of cases seen at the emergency wards was positively correlated with SO_2.
Among the elderly, SO_2, NO, NO2, and cold weather correlated significantly with admissions to emergency wards and CO correlated significantly with all asthma cases.
During the period of high SO_2 pollution, the mean number of all admissions was 7% greater than during the period of lower pollution.

The number of admissions increased during cold weather (n=4209), especially among persons who were of working age but not among children.
Regression analysis revealed that NO and O_3 were most strongly associated with asthma problems. Effects of air pollutants and cold were maximal if they occurred on the same day, except for O_3, which had a more pronounced effect after a 1-day lag. The associations between pollutants, low temperature, and admissions were most significant among adults of working age, followed by the elderly. Among children, only O_3 and NO were significantly correlated with admissions.

Author's Conclusions:

"The results of the present study, and those of some other studies, show that ambient air pollutants, at concentrations lower than the guidelines provided in many countries and by the World Health Organization, may cause an increase in the incidence of asthma."

"The frequency of admissions by emergency wards was more highly correlated with SO_2, O_3, TSP, and cold weather than was frequency of all admissions, but all admissions correlated slightly better with pollutants that primarily indicated traffic pollution on the street level. Persons with severe symptoms were first treated at the emergency wards and were later transferred to ordinary bed wards; therefore, the difference may have resulted from more severe reactions in the respiratory tract caused by SO_2, TSP, O_3, and cold weather among certain groups of severe asthmatic persons."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (Not enough info)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (+) 3 year study period. Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (No info)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval?

	. **(-) no controls**Were appropriate controls recruited? . **(n/a no blinding)**Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) . (+)Was the timing and length of follow-up appropriate? . (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	. **(n/a no controls)**Was the assessment identical for cases and controls? . (+)Were appropriate endpoints examined? . (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) . (+)Were accepted assessment methods employed? . (+)Were assessment measures sufficiently sensitive? . **(No info)**Was the nature and severity of symptoms graded against an explicitly defined scale? . (+)Did qualified personnel conduct the exams? . (+)Was the timing and frequency of assessment logical? . (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? . (-)Was individual data reported for each subject? . (n/a)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	. (+)Are the statistical methods used appropriate? . (-)Are confidence intervals reported? . (+)Did the investigator appropriately interpret the statistics? . **(some)**Are the observed trends statistically significant?
G. Interpretations:	. (+)Was the original objective addressed? . (-)Are the results novel? . (+)Was the design of the study appropriate to investigate the primary objective? . (+)Were assumptions reasonable? . (+)Are the methods reproducible? . (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? . (+)Do the conclusions make biological sense? . (-)Are the extrapolations reasonable? . (+)Is the paper in a peer-reviewed journal? . (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were some statistically significant findings; however, because the admissions correlated with other pollutants in addition to SO_2 and with cold temperatures, it is difficult to ascertain whether the asthma attacks are solely due to the SO_2 exposure. The correlations were small but remained significant after temperature standardization. This warrants further investigation into the effects of SO_2 exposure on this sensitive subgroup – persons with bronchial asthma.

There is inadequate information available on the synergistic effects with various pollutants and other factors. This study attempted to correct some of the deficiencies from past studies including an increased sample size, describing socioeconomic factors and sufficiently documenting pollutant levels.

This study suggests that ambient air pollutants increase asthma attacks at lower concentrations than previously believed. However, the results of earlier epidemiological studies are inconsistent. The cold climate also increased the effects of pollutants on the airways.

Confidence index:

This study focussed exclusively on asthma patients who were hospitalized primarily for acute asthma. The study used the World Health Organization's definition of asthma to identify patients with bronchial asthma only. Confounders were addressed and included in analysis. However, more background information is needed on subjects.

The diagnosis of bronchial asthma and asthma attacks was very thorough and based on accepted methods.

SO_2 was measured at 4 stations and there was some variability among the stations but it was not reported if this variation was significant.

The study tried to control for cold effects by standardizing for temperature and then partially correlating the data.

Positive correlation was observed after standardization for temperature. However, the correlation values were small and significance decreased. Correlations were also calculated for 1 and 2 day lags to account for possible bias introduced by the temperature.

It was difficult to interpret figures.

Consistent and inconsistent results from other studies reported. Associations reported in this study may have been due to the longer time period studied.

As was pointed out by the authors, caution should be exercised when comparing results in this study, because in this study the incidence of asthma as a disease was not considered; rather, frequency of acute attacks among persons with bronchial asthma was noted.

Potential weaknesses are: exposure assessment, misclassification of symptoms, lack of raw and individual data, and not providing any information on monitoring devices. Confidence intervals were not reported.

Review – Confidence index ranking:

Moderate

CASE-CONTROL REVIEW FORM

Author: Burnett, R.T., Smith-Doiron, M., Stieb, D., Cakmak, S., Brook, J.R.

Internal ID: 454

Title: Effects of Particulate and Gaseous Air Pollution on Cardiorespiratory Hospitalizations

Year: 1999

Abstract: We obtained data on daily numbers of admissions to hospital in Toronto, Canada, from 1980 to 1994 for respiratory, cardiac, cerebral vascular, and peripheral vascular diseases. We then linked the data to daily measures of particulate mass less than 10 microns in aerodynamic diameter (PM$_{10}$), particulate mass less than 2.5 microns in aerodynamic diameter (PM$_{2.5}$), and particulate mass between 2.5 and 10 microns in aerodynamic diameter (PM$_{10-2.5}$), ozone, carbon monoxide, nitrogen dioxide, and sulfur dioxide. Air pollution was only associated weakly with hospitalization for cerebral vascular and peripheral vascular diseases. We controlled for temporal trends and climatic factors, and we found that increases of 10 $\mu g/m^3$ in PM$_{10}$, PM$_{2.5}$, and PM$_{10-2.5}$ were associated with 1.9%, 3.3%, and 2.9% respective increase in respiratory and cardiac hospital admissions. We further controlled for gaseous pollutants, and the percentages were reduced to 0.50%, 0.75%, and 0.77%, respectively. Of the 7.72 excess daily hospital admissions in Toronto attributable to the atmospheric pollution mix, 11.8% resulted from PM$_{2.5}$, 8.2% to PM$_{10-2.5}$, 17% to carbon monoxide, 40.4% to nitrogen dioxide, 2.8% to sulfur dioxide, and 19.8% to ozone.

Objective: In the present study, we used the daily number of admissions for the 15y period between 1980 and 1994 in Toronto, Canada, to examine the association between the fine and coarse particulate mass fractions and hospital admissions for heart and lung diseases. We examined the potential confounding effect form the gaseous pollutants (i.e., CO, NO_2, SO_2 and O_3) on the association between mass and hospital visits. We also considered the relative importance of the various components of the atmospheric mix in the prediction of hospital admission rates.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
5.35 ppb (CV =110)	Data between 1980-1994 examined	Male and female	No info	Total admissions available for analysis: 449278	No info	n/a	n/a

Observations:

Statistically significant observations were not reported.

There were small percentage increases in hospital admissions for asthma, respiratory infection, and ischemic heart disease associated with increases in SO_2 exposure. The effect of SO_2 could be explained almost entirely by the other atmospheric variables. A stepwise regression model was done in which pollutants competed equally for inclusion The CO and NO_2 effects were clearly confounded, with CO remaining in the stepwise model for asthma, obstructive lung disease, and dysrhythmias whereas NO_2 was an independent predictor for ischemic heart disease.

Of the 7.72 excess daily hospital admissions in Toronto attributable to the atmospheric pollution mixture, 11.8% resulted from PM$_{2.5}$, 8.2% to PM$_{10-2.5}$, 17% to carbon monoxide, 40.4% to nitrogen dioxide, 19.8% to ozone, and 2.8% were attributable to sulphur dioxide.

Air pollution was only associated weakly with hospitalization for cerebral vascular and peripheral vascular diseases.

Author's Conclusions:

"Our data suggest that gaseous pollutants account for the majority of the health impact of the ambient air pollution mix in Toronto (i.e. effects estimates for these pollutants may serve as a better index than particulate matter for the overall impact of the air-pollution mix). Nonetheless, our results also suggest that the most accurate method of estimating the impact of a program that results in reductions in multiple pollutants is to utilize concentrations-response functions for each affected pollutant, which has been derived from a multipollutant model."

"A governmental panel was established in Ottawa, the function of which was to assess the benefits to public health of achieving selected concentrations of sulfur in gasoline. The panel modeled reductions in ambient levels of several pollutants for selected sulfur concentrations in several Canadian cities, including Toronto. Reducing the sulfur content of gasoline reduced vehicular emissions of SO_2, thus leading to a decrease in air concentrations of SO_2. We also expect that reduced fuel sulfur content will help prevent the deterioration of the catalytic converter included in newer vehicles. This approach should result in an efficient control of other gaseous emissions."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(Not enough info) Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (Not enough info)Are the subjects a representative sample of the population to which extrapolations will be made? (no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (15y study period) Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (No info)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (No controls)Were appropriate controls recruited? (no blinding)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (-) ecological study Was the timing and length of follow-up appropriate? (not applic. Ecological study)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(No controls)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom)

	(Not enough info)Were accepted assessment methods employed? (Not enough info)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (Not applic. Ecological study) Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (+)In this case, reasons for excluding certain records Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-)Are the statistical methods used appropriate? (-)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (+) some Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (-)Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

After controlling for various confounders, the authors report findings that the urban ambient atmospheric mix of pollutants in Toronto, Canada, was a risk factor for exacerbation of heart and lung diseases sufficient severe to require hospitalization. Data from this study suggest that gaseous pollutants account for the majority the health impact of ambient air pollution in Toronto.

The authors also concluded that the apparent SO_2 effect could be explained by the other variables in the pollution mix. It is difficult to separate the effects of SO_2 from the other pollutants. The conclusion that the public health impact of gaseous pollutants overshadows that of particulate matter may mean that there are important implications for estimates of the public health benefits of air pollution reduction strategies.

This study finds positive associations with both fine and coarse fractions of particulate mass.

Confidence index
The literature review is extensive.
More detail is needed on the statistical models used to analyze the data.
The extensive analysis and filtering of the large data set (approx. 449,000 admissions) may have led to spurious correlations.
Extensive filtering of the data may also lead to weaker associations. However, the authors checked the correlations between the filtered and original data and they were similar for SO_2.
The hospital discharge records contained only those admissions to acute care active treatment hospitals that were considered an emergency or urgent. Therefore, the admissions were only for the most serious cases.
Some confounders were considered.
Because of the study design, there is no baseline data or other background information on the subjects.
A comparatively small number of monitoring stations were used to measure air pollution. Data for the six cities of metropolitan Toronto were collected from 4 fixed monitoring systems. There was no information on the exact locations of the monitoring systems or the reliability of the data. There was no information provided on the homogeneity of exposure areas. Exposure misclassification is a possibility.
As addressed by the authors - The variance of each of the diseases was greater than the mean, suggesting over-dispersion relative to random Poisson variation-likely the result of temporal variation in hospital admissions. Prefiltering the admission time series for temporal variations and climate removed any over-dispersion in the data.
The risk values (calculated as T values – log relative risk/standard error) were low.
SO_2 was not considered in all of the statistical models.
Conclusions are based on the total mixture of air pollutants and not specifically on SO_2.
Good details are also provided on methods. Confidence intervals were not included and some pollutant effects were clearly confounded.
Raw and individual data were not included.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Neukirch, F., Segala, C., Le Moullec, Y., Korobaeff, M., Aubier, M.	**Internal ID:** 455
Title: Short-Term Effects of Low-Level Winter Pollution on Respiratory Health of Asthmatic Adults	
Year: 1998	
Abstract: We studied the short-term effects of Paris winter air pollution (i.e., sulfur dioxide, Black Smoke, suspended particulates with an aerodynamic diameter close to 10 μm, and nitrogen dioxide) in 40 nonsmoking mild to moderate asthmatics (52% male; mean age = 46 y; 90% treated with inhaled steroids). During a 6-mo period, subjects recorded asthma symptoms and three daily peak expiratory flow measurements. Statistical analysis (i.e., generalized estimating equation models that accounted for autocorrelation of responses, weather data, and time trends) revealed consistent and significant associations between the pollutants and asthma attacks and symptoms in the entire study group, especially in the subgroup of individuals who took inhaled beta2 agonists as needed. Pollutants correlated negatively with morning peak expiratory flow in the subgroup that took inhaled beta2 agonists as needed, and they correlated positively with daily variability in asthmatics who received regularly scheduled inhaled beta2 agonists. The effects lingered several days after exposure occurred. Low-level pollution has consistent measurable effects on nonsmoking adults who have well-treated mild or moderate asthma.	
Objective: We studied short-term effects of winter air pollution in Paris on the respiratory health of nonsmoking adult outpatients who had mild or moderate asthma, and we took meteorological factors into account.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean: 21.7 ± 13.5 $\mu g/m^3$ Range: 4.4-83.8$\mu g/m^3$	Study period: 6 months	52% male	Mean age: 46y	40	Mild to moderate asthmatics	n/a	n/a

Observations:

Statistically significant results as reported by authors:

Odds Ratios (95% Confidence Interval) of the Effects of a 50$\mu g/m^3$ increase in SO_2 on Incident and Prevalent Episodes in Asthmatics in the Entire Group

INCIDENCE

Episode	Lag(d)	SO_2 OR(95% CI)
Wheeze	5	1.66(1.01-2.70)
Nocturnal Cough	3	1.60(0.98-2.62)
	4	1.71(0.86-3.40)

	6	1.72(1.16-2.55)
	Week[+]	

[+] Mean exposure of current day and 6 previous days

PREVALENCE

Episode	**Lag(d)**	**SO$_2$** **OR (95% CI)**
Wheeze	5	1.35(1.01-1.81)
	6	1.39(1.04-1.87)
	Week	1.64(0.91-2.94)
Nocturnal Cough	6	1.34(1.00-1.79)
Shortness of breath	Week	1.56(1.06-2.32)

For asthmatics who took inhaled β2 agonists as needed, the following results were reported as significant:

Odds Ratios (95% Confidence Interval) of the Effects of a 50μg/m^3 increase in Pollutants on Incident and Prevalent Episodes in Asthmatics Taking Inhaled β2 Agonists as Needed

INCIDENCE

Episode	**Lag(d)**	**SO$_2$** **OR(95% CI)**
Asthma Attacks	6	2.19 (0.91-5.29)
Wheeze	5	1.84(1.13-3.00)
Nocturnal cough	3	2.41(1.47-3.93)
	4	2.35(0.88-6.26)
	5	1.86 (1.14-3.04)

PREVALENCE

Episode	**Lag(d)**	**SO$_2$** **OR(95% CI)**
Asthma Attacks	5	1.88(0.95-3.73)
	6	2.82(1.57-5.07)
Wheeze	5	1.51(1.02-2.23)

	6	1.57(1.06-2.32)
Nocturnal Cough	3	1.73(1.06-2.82)
	4	2.28(1.27-4.11)
	5	**1.91(1.17-3.12)**
	6	**1.91(1.17-3.12)**
Shortness of Breath	4	1.01(1.22-2.67)
	5	1.65(1.11-2.44)
	6	1.61(1.20-2.16)

In contrast, among those who received regularly scheduled inhaled β2 agonists, SO_2 was not associated with any of the symptoms.

SO_2 correlated significantly with morning PEF in asthmatics who took inhaled β2 agonists as needed. An increase of $50\mu g/m^3$ of SO_2 resulted in a maximum decrease in morning PEF of 5.5%. For the group who received regular medication, the daily PEF increased by 1-3% for each $50\mu g/m^3$ of pollutant.

Respiratory infections were also associated with SO_2 for both incident and prevalent episodes. For incidence, lags 3, 4 and 5 were significant at ORs of 3.14(1.30-7.59), 2.70(1.36-5.37), 2.79(0.95-8.21), respectively. For the mean exposure of current day and 6 previous days, the OR was 8.52 (1.20-60.5). For prevalence, lags 4, 5, 6 and weekly relationships were significant at ORs of 2.40(1.33-4.33), 2.72(1.67-4.44), 2.94(1.80-4.79) and 6.30(1.31-30.2).

Statistical analysis (i.e. generalized estimating equation models that accounted for autocorrelation of responses, weather data, and time trends) revealed consistent and significant associations between the pollutants and asthma attacks and symptoms in the entire study group, especially in the subgroup of individuals who took inhaled β2 agonists as needed. Pollutants correlated negatively with morning peak expiratory flow in the subgroup that took inhaled β2 agonists as needed, and they correlated positively with daily variability in asthmatics who received regularly scheduled β2 agonists. The effects lingered several days after exposure occurred. Low-level pollution has consistent measurable effects on nonsmoking adults who have well-treated mild or moderate asthma.

SO_2 was associated positively with both incident and prevalent episodes of wheeze and nocturnal cough, as well as with prevalent episodes of shortness of breath. Respiratory infections were associated consistently with all pollutants. Odds ratios for both incident and prevalent episodes of infections were generally greater than for asthma attacks and symptoms.

Author's Conclusions:

"Low level pollution has consistent measurable effects on nonsmoking adults who have well-treated mild or moderate asthma."

"In our study, we showed consistent relationships between the four pollutants measured and both incidence and prevalence of episodes of wheeze and nocturnal cough, and prevalence of shortness-of-breath episodes, in the study group as a whole. The subgroup of asthmatics who took inhaled β2-agonists as needed exhibited even more consistent relations—also with asthma attacks—with ORs as great as 2.8. In this subgroup, a $50ug/m^3$ increase in Black Smoke, PM_{13}, SO_2, or NO_2 concentration resulted in a -4.0% to -8.0% change in morning PEF readings. Weaker associations were found in the subgroup of asthmatics who received regularly scheduled inhaled β2-agonists; these patients were unlikely to be less susceptible to pollutants, but perhaps their symptoms were better managed by the more regular treatment. This opinion is consistent with the findings of Pope, who reported that the effects of PM_{10} pollution were greater in a school-based sample than in a patient-based sample that used asthma medication.

In this study, we have shown, for the first time in a panel of asthmatic adults that the effects of pollution lasted several days after exposure occurred. This result is consistent with the possible mechanisms indicated by experimental studies, including an inflammatory process of airways."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+/-)Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitors Was the exposure concentration measured? (+) Study period Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (No info)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls)Were appropriate controls recruited? (+) single Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (+)Are all withdrawals listed, with the reason for withdrawal?

F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (-)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (More info needed)Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (No)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The authors claim to show for the first time in a panel of asthmatic adults that the effects of pollution lasted several days after exposure occurred. This study reports measurable short-term effects of winter air pollution on asthma attacks and symptoms, as well as on PEF values, in a panel of 40 nonsmoking adults who had mild or moderate asthma. Authors conclude that asthmatics could therefore receive short-term anti-inflammatory treatment or increased doses of inhaled corticosteroids to speed resolution of exacerbations. However, the odds ratios are very low in some cases and very large in other cases, frequently with wide confidence intervals. There is also the possibility of selection bias.

Confidence index:

There was no power calculation for appropriate sample size. The original group of 46 was decreased to 40 (6 exclusions due to incomplete or absent diary data). The total sample was further decreased to N=18 (Asthmatics taking inhaled β2 agonists as needed) and N=17 (asthmatics receiving regularly scheduled inhaled β2 agonists). Five of the N=40 were excluded when the sample was divided into the two groups described above.

This study clearly outlined the recruitment process for subjects and identified the characteristics of the sample population. Reason for exclusion of subjects was also given. The study also used questionnaires and diaries to record individual subject data. However, this may have introduced bias into the process.

There is a possibility for selection bias. The subjects were all chosen by the investigators according to certain characteristics. There was no randomization.

There is no mention about the possibility of a training effect with regards to the use of the peak-flow meters.

The figure showing mean values of all of the pollutants studied is difficult to read.

It appears from the tables that only significant findings were reported. The authors are not very clear on this point.

The data for SO_2 levels and asthmatics receiving regular medication were not presented.

The odds ratios for the SO_2 effects on the entire group were relatively low. Some of the confidence intervals contained OR=1.00.

The odds ratios for the SO_2 effects on the group that received medication as needed were higher than that in the total group; however, the confidence intervals were very wide. Also, some of the intervals contain OR=1. The authors do not address this.

For the prevalence of nocturnal cough (lag5 and lag6), the odds ratios and confidence intervals are reported as being exactly the same values.

There are some errors in the descriptions of the data in the tables. For example, the table containing regression coefficients contains a note about odds ratios, but there are no odds ratios given in the table.

The table describing regression coefficients of the effects of an increase in SO_2 on peak expiratory flow variables in the two subgroups of patients reports the effects for a 1 $\mu g/m^3$ increase in SO_2; however, in the body of the paper, the authors refer to a 50 $\mu g/m^3$ increase.

The odds ratios for the effects of SO_2 and respiratory infections also contains very high OR values, but the intervals are wide, and again, one of the confidence intervals contains OR=1.

Consistency with toxicological studies is reported, but no details are given.

SO_2 was measured at 11 stations. The authors report that SO_2 values among the 11 stations were similar and therefore represent a homogenous exposure. The population studied is reported as being a homogenous population, but no further details are given.

There are limitations in the exposure assessment common to most environmental epidemiology studies.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Chew, F.T., Goh, D.Y.T., Ooi, B.C., Saharom, R., Hui, J.K.S., Lee, B.W.	**Internal ID:** 456
Title: Association of ambient air-pollution levels with acute asthma exacerbation among children in Singapore	
Year: 1999	
Abstract: BACKGROUND: Air-pollution levels have been shown to be associated with increased morbidity of respiratory diseases. METHODS: Data for ambient air-pollutant levels, meteorologic factors, and hospitalization or emergency room (ER) visits for acute asthma in Singapore children over a 5-year period (1990-4) were obtained and analyzed for associations by time-series methods. RESULTS: Throughout this period, the annual mean and 24-h mean levels for sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and total suspended particles (TSP) and maximum 1-h daily average for ozone were generally within the air-quality guidelines established by the World Health Organization (WHO). However, positive correlation between levels of each of these pollutants and daily ER visits for asthma was observed in children aged 3-12 years, but not among adolescents and young adults (13-21 years old). The association with SO_2 and TSP persisted after standardization for meteorologic and temporal variables. An adjusted increase in 2.9 ER visits for every 20 $\mu g/m^3$ increase in atmospheric SO_2 levels, lagged by 1 day, was observed on days when levels were above 68 microg/m^3. With TSP, an adjusted increase of 5.80 ER visits for every 20 μg/m3 increase in its daily atmospheric levels, lagged by 1 day, was observed on days with levels above 73 $\mu g/m^3$. Similar results were also obtained after controlling for autocorrelation by time-series analysis. CONCLUSIONS: These associations were observed even though the overall levels of all pollutants were generally within the air-quality guidelines established by the WHO. These findings suggest that asthmatic children are susceptible to increased levels of air pollutants, particularly SO_2 and TSP, although the ambient levels are generally within "acceptable" ranges.	
Objective: This study aimed to examine the relationship between the levels of ambient air pollutants and acute asthma in our population.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
SO_2 24 hr avg: 38.1 ± 21.8 $\mu g/m^3$ Range: 3.0-141.0 $\mu g/m^3$	Study duration: 5 years	Male and female	≤12y and >12-21 y	>6000 admissions and approx. 23000 ER visits recorded for asthma in children 3-12y	No info	n/a	n/a

Observations:

A significant positive correlation was found between the levels of SO_2 (increase of 20$\mu g/m^3$) (lagged by 1 or 2 days) and daily asthma ER visits in children. Adjusted increases of approximately 1.80, 2.90, and 2.64 ER visits for every 20$\mu g/m^3$ increase in daily atmospheric SO_2 levels, on the same day, lagged by 1 day and lagged by 2 days, respectively, were observed.

The regression coefficient for SO_2remained significant after successive removal of days with levels above 68 μg/m^3 (90th percentile). However, the regression coefficient was not significant after the removal of data up to the 75th percentile (50μg/m^3).
Throughout the period, the annual mean and 24h mean levels for SO_2, NO_2, and TSP and maximum 1h daily average for ozone were generally within the air quality guidelines established by the WHO. However, positive correlation between levels of each of these pollutants and daily ER visits for asthma was observed in children aged 3-12 years, but not among adolescents and young adults. The association with SO_2 and TSP persisted after standardization for meteorologic and temporal variables. With TSP, an adjusted increase of 5.8 ER visits for every 20 μg/m^3 increase in atmospheric SO_2 levels, lagged by 1 day, was observed on days when levels were above 73 μg/m^3. Similar results were also obtained after controlling for autocorrelation by time-series analysis.

Author's Conclusions:

"These associations were observed even though the overall levels of all pollutants were generally within the air-quality guidelines established by the WHO. These findings suggest that asthmatic children are susceptible to increased levels of air pollutants, particularly SO_2 and TSP, although the ambient levels are generally within "acceptable" ranges."

"In our study, we found that ambient air pollutant levels, in particular, those of SO_2, TSP, and NO_2 were consistently associated with ER visits for asthma in children. Lagged air pollutant data, particularly those lagged by 1 day, were more strongly associated with childhood asthma ER visits. In fact, the stronger lagged data have been found in various other studies and have been attributed to either delayed onset of symptomatic responses or delay in seeking treatment. Our data were also controlled for meteorologic factors, and suggested that the combinations of higher temperature and NO_2 concentrations, and wind speed and SO_2, are important predictors of ER visits for asthma in children. Inclusion of the daily total wind speed variable increased the magnitude and statistical significance of the SO_2 coefficient."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (+) controls – admission for appendicitisWere identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitorsWas the exposure concentration measured? (+) study period Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (-) Were appropriate controls recruited?

	(n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a) Was the assessment identical for cases and controls? **(more info needed)** Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(n/a mortality data)** Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+/-) Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

SO_2 levels were significantly associated with asthma ER visits in children. These findings suggest that asthmatic children may be susceptible to increased levels of SO_2 although the ambient levels are generally within "acceptable" ranges.

Confidence index:
The authors report some of the results from a threshold analysis; however, the data are not included in the study.
The introduction doesn't mention children or asthma.
The objective is not clearly stated.
Risk ratios and confidence intervals are not presented.
Some confounders not considered, such as influenza epidemics.
There were 15 monitoring stations; however, approximately 20-30% of the data were missing. There was no replacement of the missing values.
More detail is needed on the estimation of the population at risk.
The author gave a good description of the community, and reported on a wide variety of meteorological parameters. The author also chose to use daily admissions for appendicitis and urinary tract infections as control data since they are known not to be confused or associated with respiratory disease. Data for children of <3 years was not used, as the author felt that wheezing in young children could be confused with asthma.
This was an ecological study. Ecological studies are typically associated with a variety of study limitations, such as overinterpretation of data, unstandardized observation units and population in comparison, validity of the dependent variable measures and relativity of the air-pollution measurements.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Keiding, L.M., Rindel, A.K., Kronborg, D.	**Internal ID:** 457

Title: Respiratory Illnesses in Children and Air Pollution in Copenhagen

Year: 1995

Abstract: The relationship between air pollution and the daily number of contacts (i.e., telephone calls and home visits) with or at Copenhagen Emergency Medical Service for children with and without respiratory illnesses was studied during a 91-d period (i.e., January 14, 1991, to April 14, 1991). A total of 12,132 contacts occurred. Diagnoses, which were recorded on the invoices for 5,307 contacts, revealed that 3,974 contacts were the result of respiratory illnesses. Regression analysis was used to investigate the short-term relationship between pollutants (i.e., carbon monoxide, nitric oxide, nitrogen dioxide, NOx, sulfur dioxide, ozone, and black smoke), measured at monitoring stations, and both the number of all contacts for children and the number of contacts for children with respiratory illnesses. Temperature and systematic effects that were the result of holidays and weekends were controlled for, after which only nitric oxide and NOx were associated significantly with the number of contacts for children who had respiratory illnesses. Nitric oxide and NOx, as indicators of traffic pollution, appeared, at low levels, to slightly exacerbate respiratory illnesses among children.

Objective: Our aim in this study was to analyze, over time, the relationship between levels of air pollutants in Copenhagen and respiratory illnesses in children.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Range of daily averages: 0-100μg/m^3	Study period: January 14, 1991-April 14, 1991	Male and female	0-15y	Of 65,297 children living in the two cities when the study started 12,132 contacts occurred Current diagnoses available for only 5307 contacts Approximately 74.9% (3974) were respiratory illness	Varied	n/a	n/a

Observations:

Black smoke and SO_2 were averaged from three stations, and O_3 and CO were averaged from two monitoring stations. Levels of all pollutants, except O_3, were high during the beginning of the study period; however, a decrease followed, and levels became almost stationary thereafter. In general, the pollution measurements showed great daily variations. Highly significant negative correlations were obtained when O_3 was compared with BS, CO, SO_2, NO, and NOx. No correlation was found for NO_2 and SO_2, whereas highly significant positive correlations were found between pairs of other pollutants.

Regression analysis was used to investigate the short-term relationship between pollutants measured at monitoring stations, and both the number of all contacts for children and the number of contacts for children with respiratory illnesses. Temperature and systemic effects that were the result of holidays and weekends were controlled for, after which only nitric oxide and Nox were associated significantly with the number of contacts for children who had respiratory illnesses. There were no significant associations with SO_2 levels and the total number of contacts on the same day or the following day (p value .68). There were no significant associations between current day and following day SO_2 levels and contacts with registered respiratory illness.

Author's Conclusions:

"It was not surprising that, in our type of study, no such relationship was found for SO_2, given the relatively low levels of this pollutant in Copenhagen.
Our results, and the findings of other epidemiologic studies, allow us to conclude that air pollution at low levels (compared with the World Health Organization's air quality guidelines) minimally exacerbates respiratory illnesses in children."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(Not enough information) Is the number of subjects sufficient to produce statistically significant results? (-)Collected but not provided in study Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls)Were identical 'criteria for exclusion' applied to both cases and controls? (-)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Fixed monitoring sitesWas the exposure concentration measured? (+) 91 days Is the exposure duration known?
C. Equipment:	(+)Was a full description of the monitoring devices provided? (No info)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls)Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls)Was the assessment identical for cases and controls? (Not enough information provided)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom)

	(Not enough info) Were accepted assessment methods employed? (-)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (-)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (-)Are confidence intervals reported? **(Not enough information provided)**Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(n/a no specific conclusions re SO_2)**Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of Findings:

There were no statistically significant findings relating SO_2 levels and respiratory illness in this study. This does not necessarily mean a relationship does not exist. There are some problems with the study design that may have affected the observation of an association between SO_2 and respiratory illness.
The levels of air pollution measured and the variations of pollution were typical for Copenhagen for the season in question, with the exception of the first few days of the period, during which the levels of pollution were unusually high.

Confidence Index:

Inadequate analysis and discussion of results. For the pollutant analysis, there is a figure showing varying levels of SO_2 over the 91 d time period, and a figure showing number of contacts during the study period, but otherwise, just a table of p values. There was no information on average levels of SO_2, risk or confidence intervals.

Approximately 27days of observations were excluded from the 91 day study. These data were excluded because of large deviations in the data. Therefore, only 64 days were available for analysis.

The literature review is inadequate.

Contacts with the Copenhagen Emergency Medical Service were chosen to be a measure of exacerbations of respiratory illness in children. Primary healthcare contacts were not included.

The contacts included phone calls to medical doctors and home visits.

There was an influenza epidemic at the beginning of the study which may have confounded the results.

The SO_2 levels varied among monitoring stations. However, the authors do not give any details as to the amount of variation.

There is limited description of the statistical analysis.

The monitoring stations were located in areas of heavy traffic loads on main streets at three meters off the ground and therefore may not represent true personal exposure. This study cannot be used directly for dose-response calculations in light of the fact that monitoring stations are situated where there can be incidences of high traffic of which children are not likely to be exposed to over prolonged periods.

The total number of registered contacts was assumed to be almost complete; however, the percentage of invoices giving a diagnosis was not necessarily complete. Physicians were not always likely to give details of diagnosis, especially during busy times.

Contacts with general practitioners during regular business hours were not included.

This study provided graphical representation of pollutant levels but did not provide summary information on the parameters.

Confidence intervals were not reported nor was detailed information on monitoring stations.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Simpson, R.W., Williams, G., Petroeschevsky, A., Morgan G., Rutherford, S.	**Internal ID:** 458

Title: Associations between Outdoor Air Pollution and Daily Mortality in Brisbane, Australia

Year: 1997

Abstract: The results of several studies have indicated significant associations between daily mortality and air pollution, with little evidence of a threshold. In the current study, the authors examined daily mortality during the period 1987-1993 for the Brisbane region, which is the fastest-growing urban region in Australia (annual average concentration of particulate matter less than 10 µm in diameter = 27 µg/m^3, maximum hourly sulfur dioxide level = 60 ppb, and maximum daily ozone hourly level = 118 ppb). The authors conducted a general estimating equation analysis, and they used autoregressive Poisson models for daily mortality to examine associations with air pollution variables. The authors used research methods developed in the Air Pollution on Health, European Approach (APHEA), project to control confounding effects of weather and temporal trends. The air pollutants examined included particulate pollution (measured by nephelometry [bsp data]), sulfur dioxide, ozone, and nitrogen dioxide. The results indicated that the associations between total daily mortality and particulate levels found in studies in the United States and other countries may be applicable in Brisbane, Australia. Ozone levels were also associated significantly with total daily mortality. There was little evidence of interaction between the ozone effects (mainly in summer) and particulates or with sulfur dioxide and nitrogen dioxide. The associations between pollutants (ozone, bsp) and daily mortality were significant only for individuals who were older than 65 y of age; positive associations were also found with cardiovascular disease categories, and the regression coefficients--when significant--were higher than those for total mortality. The results indicated a possible threshold for ozone levels, but a similar result for particulate levels was not apparent.

Objective: In the present study, we used the APHEA (Air Pollution on Health: European Approach) protocol to examine associations between daily mortality and air pollution levels monitored in Brisbane, Australia.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
SO_2 (ppb) 24-h average: All year: Mean 4.2 Range 0-35.5 Summer: Mean 3.9 Range 0-20.6 Winter: Mean 4.5 Range 0-35.5	Study period: January 1, 1987-October 31, 1993	Male and female	No info	N=2496 days of observation	No info	n/a	n/a

SO_2 (ppb) daily 1-h max: All year: Mean 9.6 Range 0-59.5 Summer: Mean 8.5 Range 0.1-55.0 Winter: Mean 10.7 Range 0-59.5							

Observations:

The results for SO_2 were not significant.
Relative risks for SO_2 were not presented due to insignificance of results.
The results indicated that the associations between total daily mortality and particulate levels found in studies in the United States and other countries may be applicable in Brisbane, Australia.
There was little evidence of interaction between the ozone effects and particulates or with sulfur dioxide and nitrogen dioxide.
Maximum 1h and 24h bsp, as well as maximum 1h and 8h average O_3 levels were associated significantly with total daily mortality, especially in summer. These associations did not remain significant in the respiratory mortality category, but the daily total number of deaths from respiratory illness was very low. The positive associations persisted for the cardiovascular mortality category, but they were significant only for maximum 1h bsp data.

Author's Conclusions:

Sulfur dioxide levels did not appear to be a confounding effect; they were likely too low for this to have occurred.

"The results indicated significant associations between O_3 and particulate pollution, and total daily mortality in Brisbane. The association for particulates was similar in magnitude to that found in foreign studies. Sulfur dioxide levels did not appear to be a confounding effect; they were likely too low for this to have occurred. The particulate data did not support a threshold effect, but the O_3 effect did appear to have a threshold, albeit low. We did not note any interaction effects between the pollutants; the impact of O_3 and particulate pollution appeared additive. There was an absence of any confounding effects for very hot days or for "natural" events that might have distorted the results. The associations were significant only for elderly people; we found positive, but generally not significant, associations for cardiovascular categories of mortality."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	. (+)Is the number of subjects sufficient to produce statistically significant results? . (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate

	for the objective of the study? (Not enough info) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls)Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitorsWas the exposure concentration measured? (+) 2496 days Is the exposure duration known?
C. Equipment:	(+)Was a full description of the monitoring devices provided? (-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls)Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.,* single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (+)Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (-)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel?

	(+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a no specific conclusions re SO_2) Do the conclusions make biological sense? (no extrapolations made) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no statistically significant findings relating SO_2 levels. This may have been due to the very low levels of SO_2 during the study period or the fact that the exposure levels were calculated from fixed monitoring stations (only 2) covering a large area.

Results from this study suggest a significant association between O_3 and particulate pollution and total daily mortality in Brisbane. Sulfur dioxide levels did not appear to have a confounding effect, but may have been too low to impact the study. All findings were significant for elderly people only which is to be expected, given the reduced immune capabilities of the elderly population.

Positive associations were also found with cardiovascular disease categories, and the regression coefficients—when significant—were higher than those for total mortality. More study needs to be done to examine biological plausibility, examine the question of coherence, and measure total exposure (indoor and outdoor).

Confidence index:

Although there were no statistically significant findings in this study, it was generally well done with an extensive discusson of the methods. However, the results section is very brief. Most confounding variables were considered with extensive detail on the methods used to control for the confounders.

The SO_2 levels were monitored at only 2 sites covering a very large area.

The discussion and conclusions focused on other pollutants with a more significant association.

This study listed very few of the characteristics associated with the sample subjects. As stipulated by the APHEA protocol, the study set up monitoring sites near an urban traffic road and near a "background" station. However, the study used the bare minimum number of monitoring sites, which would have been unlikely to properly represent the air pollution across such a large study area.

Though no raw data was given, results were well-presented in many tables. This study follows the protocols of the APHEA studies done in Europe. Limitations on exposure assessment were minimized as much as possible.

Review – Confidence index ranking:

Moderate to low

CASE-CONTROL REVIEW FORM

Author: Sunyer, J., Ballester, F., Le Tertre, A., Atkinson, R., Ayres, J.G., Forastiere, F., Forsberg, Bertil, Vonk, J.M., Bisanti, L, Tenias, J.M., Medina, S., Schwartz, J., and Katsouyanni, K.	**Internal ID:** 459
Title: The association of daily sulfur dioxide air pollution levels with hospital admissions for cardiovascular diseases in Europe (The APHEA-II study)	
Year: 2002	
Abstract: The objective of this study is to assess the short-term effect of sulfur dioxide (SO_2) air pollution levels on hospital admissions for cardiovascular diseases. Daily mean hospital admissions for cardiovascular diseases, ischemic heart diseases (IHDs), and stroke in seven European areas (the cities of Birmingham, London, Milan, Paris, Rome, and Stockholm, and in The Netherlands) participating in the multicenter European study of air pollution (Aphea-II), were measure. Time series analysis of daily hospital admission counts was performed using poison autoregressive models. A summary regression coefficient for all cities was provided. Daily numbers of all cardiovascular admissions except stroke, and particularly IHDs, rose significantly with an increase of daily SO_2 levels of the same day and day before. After adjusting for PM_{10} (i.e. particles with size <10um), the association of SO_2 with IHD admissions remained significant (i.e. and increase of 0.7%; 95% confidence interval = 0.1-1.3, per each 10μg/m^3 increase of SO_2) among subjects younger than 65 years, but not among subjects older than 65. In the older group the increase was only significant for particles (1.3%; CI 0.7-1.8, per each increase in 10μg/m^3 of PM_{10}). This study provides new evidence for the effects of urban air pollution on cardiac disease in Europe, and suggests that SO_2 pollution may play an independent role in triggering ischemic cardiac events. From a Public Health perspective these results suggest that reduction in SO_2 levels in European cities could imply a reduction of admissions for IHDs.	
Objective: To study the short-term effect of SO_2, after adjusting for particles and other air pollutants, on hospital admissions for cardiovascular diseases.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
5-21 μg/m^3	daily/7 years (1990-1996)	male and female	all	Seven European cities	Cardiovascular disease and stroke	-	-

Observations:

Daily numbers of all cardiovascular admissions except stroke, and particularly IHDs, rose significantly with an increase of daily SO_2 levels of the same day and day before. After adjusting for PM_{10} (i.e. particles with size <10μm), the association of SO_2 with IHD admissions remained significant (i.e. and increase of 0.7%; 95% confidence interval = 0.1-1.3, per each 10μg/m$_3$ increase of SO_2) among subjects younger than 65 years, but not among subjects older than 65. In the older group the increase was only significant for particles (1.3%; CI 0.7-1.8, per each increase in 10μg/m^3 of PM_{10}).

*Statistically significant

Author's Conclusions:

"The present analysis shows that from a public health perspective, urban atmospheres in Europe are associated with admissions for IHD, and that SO_2 is a surrogate of these mixtures, in some cases in a stronger way than particles. Therefore, these results suggest that reduction in SO_2 levels in European cities could

imply a reduction of admissions for IHDs."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (+)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a)Were identical 'criteria for exclusion' applied to both cases and controls? (n/a)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Was the exposure concentration measured? (+)Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(n/a)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a)Were appropriate controls recruited? (n/a)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (n/a)Was the timing and length of follow-up appropriate? (n/a)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (+)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+/-)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (n/a)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+/-)Are the statistical methods used appropriate?

	(-)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (+)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (+)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? (+)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (+)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study attempted to study the short-term effect of SO_2 after adjusting for particles and other air pollutants. Results suggest that reduction in SO_2 levels in European cities could imply a reduction of admissions for IHDs.

Confidence index:

Data collection may have been a problem in this study. Not all cities were able to provide complete data. Also, authors did not incorporate the classical risk factors for cardiovascular diseases nor were they corrected for in the statistical analysis (confounding).

This study utilized seven European areas. While most data was provided from daily admission registries, several cities utilized emergency admissions, while other used general admissions. The Netherlands could not provide information for all cardiac causes. Use of different data sets may have incorporated an element of bias into the study.

There are limitations in the data for both the exposure and the outcomes.

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM

Author: Meng, Z, Zhang, B., Bai, J., Geng, H., Liu, C. **Internal ID:** 460

Title: Oxidative Damage of Sulfur Dioxide Inhalation on Stomachs and Intestines of Mice

Year: 2003

Abstract: Effects of exposure to sulfur dioxide (SO_2) on levels of thiobarbituric acid-reactive substances (TBARS), levels of reduced glutathione (GSH), and the activities of Cu, Zn-superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) were investigated in stomachs and intestines of Kunming albino mice of both sexes. SO_2 exposure at different concentrations (22, 56, and 112 mg/m^3) was administered to the animals of SO_2 groups in the exposure chambers for 6 h/day for 7 days, while control groups were exposed to filtered air under the same condition. Our results show that SO_2 caused lipid peroxidation and changes of antioxidative status in stomachs and intestines of mice. Exposure to SO_2 at all concentrations tested caused significantly the increase of TBARS levels in stomachs and intestines of mice. For the stomachs, activities of these antioxidant enzymes and levels of GSH were significantly unaltered by SO_2 at low concentrations, except significant increase of SOD activity in the stomachs of male mice. However, higher SO_2 caused the significant increases of CAT activities and the significant decreases of GSH levels and activities of SOD and GPx. For intestines, SO_2 at all concentrations tested decreased significantly activities of SOD relative to control animals; SO_2 caused the decreases of GPx activities, but only the decreases of GPx activities caused by SO_2 exposures at higher concentrations (56 and 112 mg/m^3) were statistically significant. SO_2 at all concentrations tested tended to increase the CAT activities in a dose-dependent manner, but the decreases of CAT activities caused by higher SO_2 were significant. SO_2 at all concentrations tested decreased significantly levels of GSH in intestines of both sexual mice. These results lead to conclusion that SO_2 exposure can caused oxidative damage to stomachs and intestines of mice, and SO_2 is a toxic agent to stomachs and intestines of mammals, not only to respiratory system. Further work is required to understand toxicological role of SO_2 on multiple or even all organs in human and animal.

Objective: In the present study, the stomachs and intestines of mice were assessed for oxidative stress and antioxidant status caused by SO_2 inhalation at various concentrations.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
3 groups exposed to: 22±2, 56±3 and 112±8 mg/m^3 3 control groups exposed to	6h /day for 7 days	Mice	Kunming albino	5 wks	50% M 50%F	120 6 groups of 20 which were further subdivided into two groups of 10 (10 female and 10 male per experiment)		No observed adverse effect level	Lowest observed adverse effect level

filtered air									

Observations:

SO_2 inhalation at all concentrations tested caused decrease of GPx activities in stomachs from mice of both sexes, but at low concentrations (22 mg/m^3) the decrease of GPx activities is not statistically significant. Low SO_2 concentrations (22mg/m^3) caused increase of SOD activities in stomach from mice of both sexes. Higher SO_2 (56 mg/m^3 and 112 mg/m^3) caused significant decrease of SOD activities in stomachs from mice of both sexes. For CAT, SO_2 at all concentrations tested caused an insignificant increase of CAT activities in stomachs from mice of both sexes.

SO_2 exposure at all concentrations tested caused significant increases of TBARS levels in stomachs from mice of both sexes. SO_2exposure at all concentrations tested caused a decrease of GSH levels in stomachs from mice of both sexes, but only the decrease of GSH levels in stomachs of mice caused by SO_2 at high concentration (112 mg/m^3) was statistically significant.

SO_2inhalation at all concentrations tested caused significant decreases of SOD activities in intestines in mice of both sexes. SO_2 exposures at all concentrations tested also caused decreases of GPx activities in intestines from mice of both sexes, but the decreases induced by low SO_2 (22mg/m^3) were statistically insignificant. SO_2 at all concentrations tested caused increases of CAT activities in intestines from mice of both sexes, but only the increases of CAT activities caused by higher SO_2(56, 112 mg/m^3) in intestines from male mice were statistically significant.

SO_2exposure at all concentrations tested caused significant decreases of GSH levels in intestines from mice of both sexes. SO_2 inhalation at higher concentrations (56, 112 mg/m^3) caused significant increases of TBARS levels in intestines from mice of both sexes. However, the changes of TBARS levels caused by lower SO_2 exposure (22mg/m^3) in intestines from mice of both sees were not statistically significant.

Mean body weight gain was not different in mice exposed to SO2 with respect to their corresponding control groups during the experimental period, although the final weights of both control and SO_2-exposed groups were significantly increased relative to the beginning values.
No deaths were observed after treatment. Necropsy revealed no treatment effect in any organ examined.

*Statistically significant

Author's Conclusions:

"In summary, results from the present research show that 1. SO_2exposure resulted in a significant increase in the lipid peroxidation process in the stomachs and intestines of mice of both sexes, accompanies by changes of antioxidant status in these organs; 2. effects of SO_2 on living organisms are many-sided; and 3. SO_2 is also a toxic agent to stomach and intestine, not only to respiratory system. Further work is required to understand the toxicological role of SO_2 on multiple or even all organs in mammals."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results?

	↳ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? **(+)** a) Specie ↳ *Guideline: Rat, or justification for use of another mammalian specie.* **(+) 5 wks)** Age ↳ *Guideline: Adult, i.e.,8-12 weeks.* **(+)** c) Sex **(no info)** d) Pre-test health ↳ *Guideline: Females should be nulliparous and non-pregnant.* **(+)** e) Weight variation ↳ *Guideline: Below 20% variation* **(not stated)** f) Source ↳ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	**(-)** Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? **(+)** Was there a gradient of exposure levels? ↳ *Guideline: Three exposure levels in addition to control.* **(+)** Were the chosen exposure levels appropriate to investigate the primary objective? **(+)** Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(+)** Were both nominal and actual exposure concentrations recorded? **(+)** Was the duration of exposure appropriate to investigate the primary objective? **(+)** Was the duration of exposure precisely defined? **(no info)** If exposure chambers were employed, were animals exposed during the equilibration period? ↳ *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(+)** Was the mode of administration appropriate? **(+)** Was the frequency of administration appropriate to investigate the primary objective? **(+)** Was evidence provided to justify the dosing regime? **(not stated)** Were exposure concentrations, airflow, temperature, and humidity

	monitored continuously?
C. Housing/Feeding:	**(+)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(+) number in housing cage given but not number in chamber** Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* **W(not stated)** as the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* **(No)** Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* **(+)** Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(+) only dimensions** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(+-)** Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(+)** Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* **(+) diet** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(+)** Were test animals randomly assigned to exposure groups? **(-)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(+)** Was a control group assayed? **(+) more detail needed** Is the control regime appropriate?

	(-) animals sacrificed Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	**(+) appropriate for the objective** Were appropriate endpoints examined? **(+)** Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(+)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(+)** Was the timing and frequency of assessment logical? **(-)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each test animal?
G. Data analysis:	**(+)** Are the statistical methods used appropriate? **(+)** Are confidence intervals reported? **(+)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
H. Interpretations:	**(+)** Was the original objective addressed? **(+)** Are the results novel? **(+)** Was the design of the study appropriate to investigate the primary objective? **(more details needed)** Were assumptions reasonable? **(-)** Are the methods reproducible? **(+)** Did either the study design or the statistical analysis limit confounding variables? **(+) for mice** Do the conclusions make biological sense? **(-)** Are the extrapolations reasonable? **(+)** Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The results in this study suggest that SO2 inhalation increased levels of lipid peroxidation in stomachs and intestines in mice of both sexes, accompanied by significant changes of activities of Cu,Zn-SOD and Se-dependent GSH-Px and levels of GSH in the organs tested. The findings provided evidence as the toxicological role of SO_2 inhalation on these organs of mice. The concentrations used are much higher than a human would be exposed to in the general population.

SO_2 exposure in this study decreased activities of SOD and GPx from mice of both sexes relative to control animals, while the decreases of GPx activities caused by low SO_2 were not statistically significant. SO_2 inhalation also tended to increase CAT activities in intestines from mice of both sexes, but the increases of CAT activities were statistically significant in only higher SO_2 exposure groups. These results show that SO_2 caused changes of SOD, GPx, and CAT activities in intestines of mice in a dose-dependent manner.

Confidence index:

The study was generally well done.

Details are provided on the housing of the mice, but not how they are grouped in the exposure chambers.

There is limited information provided on the equipment, in particular the sensitivity of the monitoring device.

There is no information on the source of the mice or the type of food.

More details are needed on the control regime.

There is no information on the pre-test health of the mice.

Justification is provided for exposure levels, choice of animal and exposure duration.

This study employed a satisfactory number of subjects and limited possible confounding factors such as sex, weight and species. Furthermore, mice were randomly assigned groups, and some were utilized as the control group. Based on the detail given, the methods would be reproducible, although the translation into English is stilted at times. The authors specify the feeding schedule of the animals and outline the environmental conditions that the mice were subjected to (temperature, relative humidity). The authors make use of appropriate statistical analysis, using a Student's t-test to assess differences between SO_2-exposed groups and the corresponding control groups.

Confidence intervals were not reported.

Review – Confidence index ranking:

Moderate

CASE-CONTROL REVIEW FORM

Author: Venners, S.A., Wang, B. Peng, Z., Xu, Y., Wang, L., Xu, X.	**Internal ID:** 461

Title: Particulate Matter, Sulfur Dioxide, and Daily Mortality in Chongqing, China

Year: 2003

Abstract: In 1995, daily mortality in a district of Chongqing, China, was analyzed from January through December for associations with daily ambient sulfur dioxide and fine particles (airborne particles with diameters less than or equal to 2.5 microm; $PM_{2.5}$. The mean concentration of $PM_{2.5}$ was 147 µg/m^3 (maximum, 666 µg/m^3), and that of SO_2 was 213 µg/m^3 (maximum, 571 µg/m^3). On average, 9.6 persons died each day. We used a generalized additive model using robust Poisson regression to estimate the associations of mean daily SO_2 and $PM_{2.5}$ with daily mortality (on the same day and at lags up to 5 days) adjusted for trend, season, temperature, humidity, and day of the week. The relative risk of mortality associated with a 100 microg/m^3 increase in mean daily SO_2 was highest on the second lag day [1.04; 95% confidence interval (CI), 1.00-1.09] and the third lag day (1.04; 95% CI, 0.99-1.08). The associations between daily mortality and mean daily $PM_{2.5}$ were negative and statistically insignificant on all days. The relative risk of respiratory mortality on the second day after a 100 µg/m^3 increase in mean daily SO_2 was 1.11 (95% CI, 1.02-1.22), and that for cardiovascular mortality was 1.10 (95% CI, 1.02-1.20). The relative risk of cardiovascular mortality on the third day after a 100 µg/m^3 increase in mean daily SO_2 was 1.20 (95% CI, 1.11-1.30). The relative risks of mortality due to cancer and other causes were insignificant on both days. The estimated effects of mean daily SO_2 on cardiovascular and respiratory mortality risk remained after controlling for $PM_{2.5}$.

Objective: A collaborative epidemiologic investigation was conducted in 1995 in the Shi-Zhong District of Chongqing, China, to measure ambient air pollution and to assess its health effects. In this report we present the association of $PM_{2.5}$ and SO_2 with daily mortality.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean SO_2: 213 µg/m^3 (range 32.0-571.0 µg/m^3)	Study period 365 days	Male and female	No info	Total Mortality: 9.6/day (mean) Respiratory: 2.1/day Cardiovascular: 2.9/day Cancer: 0.4/day Other: 4.2/day			

Observations:

The regression coefficients for increased risk of mortality due to increased SO_2 were positive at all lags and highest on the second and third lag days. Significant positive associations were observed between $100\mu g/m^3$ increases in SO_2 and cardiovascular mortality on the second and third lag days and between SO_2 and respiratory mortality on the second lag day.

The estimated defects of a $100\mu g/m^3$ increase in mean SO_2 concentrations on total and cause specific mortality on second and third lag days:

	RR (95% CI) of mortality	
Cause of mortality	**Second lag day**	**Third lag day**
Total	1.04 (1.00-1.09)	1.04(0.99-1.08)
Respiratory	1.11 (1.02-1.22)*	1.00(0.91-1.10)
Cardiovascular	1.10 (1.02-1.20)*	1.20 (1.11-1.30)**
Cancer	1.02 (0.79-1.02)	0.94 (0.74-1.18)
Other	1.03 (0.97-1.10)	0.99 (0.93-1.06)

The associations between daily mortality and mean daily $PM_{2.5}$ were negative and statistically insignificant on all days. The estimated effects of mean daily SO_2 on cardiovascular and respiratory mortality risks remained after controlling for $PM_{2.5}$.

*Statistically significant

Author's Conclusions:

"We conclude that, in this population, daily ambient SO_2 concentrations were positively and significantly associated with population risks of cardiovascular and respiratory mortality, even after controlling for daily ambient $PM_{2.5}$. Regardless of whether SO_2 directly increased the risk of mortality in this population or is a correlated indicator of the biologically relevant air pollution mixture (that might or might not include SO_2), our results support the conclusion that consistent associations between SO_2 and daily mortality found in previous Chinese studies appear not to have been confounded by unmeasured fine particles."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls)Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)(fixed monitors) Was the exposure concentration measured? (+)365d study period Is the exposure duration known?

C. Equipment:	(+)Was a full description of the monitoring devices provided? **(not provided)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)**Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)**Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (+)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? **(n/a mortality data)**Was individual data reported for each subject? **(+) exclusions given**Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+/-)Were assumptions reasonable? (-)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? (+)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? **(No)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study demonstrated positive associations between daily ambient SO_2 concentrations and population risk of mortality, especially that from respiratory or cardiovascular events. However, the relative risks were all very close to 1.00. Also, when the data were reanalyzed after excluding 3 days with extremely high mortality counts and pollution concentrations on each of these days and the 5 days before each, the associations between SO_2 and mortality due to cardiovascular and respiratory causes on the second lag day were no longer statistically significant. In contrast to the change in the significance of effect estimates on the second lag day, those on the third lag day were unaffected by this exclusion. Due to the few peak mortality days in the data, no conclusions can confidently be made about these peak pollution days.

Recent refinements in investigation suggest that associations between SO_2 and mortality in past Chinese studies might have been confounded.

There was no observation of association between daily ambient $PM_{2.5}$ concentrations and any cause of mortality. The associations found between daily ambient SO_2 and mortality were unchanged after controlling for $PM_{2.5}$.

Confidence index:

Both consistency and inconsistency reported with other studies. This study differs somewhat from the other studies due to the high density of people living near the ambient monitors.

Good description of monitoring methods and quality control and quality assurance methods. However, this study utilized only two monitoring stations, which may not have been adequate for the large sample area involved.

Cancer deaths were included in total mortality. However, this inclusion doesn't apply to acute exposure of SO_2.

No detail on what other causes of deaths included.

A potential limitation was partial ascertainment of mortality because of death reports being filed several months after the actual time of death. The authors tried to account for this, but there was no way of knowing how many cases were delayed in reporting. The authors tried to account for this by reanalyzing the data for the first six months assuming that this time would be a more accurate assessment of mortality occurring during 1995. It is reported that by restricting the data to the first 6 months, the estimated relative risk of cardiovascular mortality due to SO_2 increased on the second lag day, whereas all other associations remained within the 95% CIs of the original analysis. However, looking at the tables of results, the RR reported for the cardiovascular mortality for the first six months of the year is 1.23 and the CI is (1.11-1.17). The RR does not fit within the CI. This could be a printing error in the article?

Standard deviations were not included.

This study characterized the sample as much as possible, indicating that coal was the dominant energy source and that many heavy industries were located in Chongqing at the time of the study.

The study design did not appear to fully limit confounding variables. In this case, the main confounding factor was unmeasured fine particles.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: **Yu, O., Sheppard L., Lumley, T., Koenig, J.Q., Shapiro, G.G.** | **Internal ID:** 462

Title: **Effects of Ambient Air Pollution on Symptoms of Asthma in Seattle-Area Children Enrolled in the CAMP Study**

Year: **2000**

Abstract: We observed a panel of 133 children (5-13 years of age) with asthma residing in the greater Seattle, Washington, area for an average of 58 days (range 28-112 days) during screening for enrollment in the Childhood Asthma Management Program (CAMP) study. Daily self-reports of asthma symptoms were obtained from study diaries and compared with ambient air pollution levels in marginal repeated measures logistic regression models. We defined days with asthma symptoms as any day a child reported at least one mild asthma episode. All analyses were controlled for subject-specific variables [age, race, sex, baseline height, and FEV_1 PC_{20} concentration (methacholine provocative concentration required to produce a 20% decrease in forced expiratory volume in 1 sec)] and potential time-dependent confounders (day of week, season, and temperature). Because of variable observation periods for participants, we estimated both between- and within-subject air pollutant effects. Our primary interest was in the within-subject effects: the effect of air pollutant excursions from typical levels in each child's observation period on the odds of asthma symptoms. In single-pollutant models, the population average estimates indicated a 30% [95% confidence interval (CI), 11-52%] increase for a 1-ppm increment in carbon monoxide lagged 1 day, an 18% (95% CI, 5-33%) increase for a 10-$\mu g/m^3$ increment in same-day particulate matter < 1.0 microm ($PM_{1.0}$), and an 11% (95% CI, 3-20%) increase for a 10-$\mu g/m^3$ increment in particulate matter < 10 μm (PM_{10}) lagged 1 day. Conditional on the previous day's asthma symptoms, we estimated 25% (95% CI, 10-42%), 14% (95% CI, 4-26%), and 10% (95% CI, 3-16%) increases in the odds of asthma symptoms associated with increases in CO, $PM_{1.0}$, and PM_{10}, respectively. We did not find any association between sulfur dioxide (SO_2) and the odds of asthma symptoms. In multipollutant models, the separate pollutant effects were smaller. The overall effect of an increase in both CO and $PM_{1.0}$ was a 31% (95% CI, 11-55%) increase in the odds of symptoms of asthma. We conclude that there is an association between change in short-term air pollution levels, as indexed by PM and CO, and the occurrence of asthma symptoms among children in Seattle. Although PM effects on asthma have been found in other studies, it is likely that CO is a marker for vehicle exhaust and other combustion by-products that aggravate asthma.

Objective: In this study we attempted to assess the effects of air pollution on daily symptoms of asthma aggravation in children on an individual level in Seattle, Washington.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Range: 1-21ppb	Study period: November 1993 through August 1995	Male = 84 Female=49	5-12 years	133	History of chronic mild to moderate asthma	n/a	n/a

Observations:

Same day SO_2 levels had the strongest effects on asthma symptoms after controlling for subject-specific variables and time-dependent confounders. A significant association between SO_2 and asthma symptoms was not found.

Percentiles of SO_2 and estimates of odds rations of asthma symptoms for a short-term 10 ppb increase in SO_2

Exposure	**Odds Ratio (95% CI)**
Same day	1.07 (0.90-1.27)
1 day lag	1.07(0.90-1.28)
2 day lag	1.00 (0.83-1.20)

"In single-pollutant models, the population average estimates indicated a 30% increase for a 1-ppm increment in carbon monoxide lagged 1 day, an 18% increase for a 10 $\mu g/m^3$ increment in PM_{10} lagged 1 day. Conditional on the previous day's asthma symptoms, an estimated 25%, 14%, and 10% increases in the odds of asthma symptoms associated with increases in CO, $PM_{1.0}$ and PM_{10} respectively. No associations were found between SO_2 and the odds of asthma symptoms. In multipollutant models, the separate pollutant effects were smaller. The overall effect of an increase in both CO and $PM_{1.0}$ was a 31% increase in the odds of symptoms of asthma."

*Statistically significant

Author's Conclusions:

There were no specific conclusions pertaining to SO_2 exposure.

"We conclude that there is an association between change in short-term air pollutions levels, as indexed by PM and CO, and the occurrence of asthma symptoms among children in Seattle. Although PM effects on asthma have been found in other studies, it is likely that CO is a marker for vehicle exhaust and other combustion by-products that aggravate asthma."

"We found that symptoms of asthma aggravation in a population of children with mild to moderate asthma were associated with air pollutants known to be emitted from combustion sources. The children in this study were a selected group and were all under a physician's care for asthma; thus they probably differ in this respect from the population of children reporting to hospital emergency departments for asthma symptoms. These results for daily symptoms complement the other Seattle-area studies that found air pollution health effects for emergency department visits and hospital admissions. Taken together, these studies suggest that the health effects among asthmatics from short-term changes of air pollution levels are an important public health problem."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(Not enough information provided)Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls)Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure	(+) fixed monitors Was the exposure concentration measured? (+) Study period Nov 1993 – August 1995 Is the exposure duration known?

conditions:	
C. Equipment:	(+)Was a full description of the monitoring devices provided? **(Not given)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(+)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)**Were appropriate controls recruited? (+)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (-)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? **(some)**Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? **(-) Reasons not given for exclusions** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (-)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(n/a no conclusions re SO_2)** Do the conclusions make biological sense? **(n/a no extrapolations made)** Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal?

	. (No)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no significant associations observed between SO_2 and asthma symptoms. The SO_2 levels were reported to be very low.

In this panel study of children with asthma, increased exposure to air pollutants, specifically CO and PM, was associated with increased odds of at least one mild asthma symptom.

Confidence index:

The literature review was extensive.

Blinding was possible because the children were not aware that their symptom information was going to be used for an air pollution study. They were taking part in a study examining use of medication in asthmatics.

The symptom data used were obtained from the children while they were not using medication, so there is no confounding from medication use.

There are no details as to how the 133 children were chosen from the group of 144.

The average number of days of diary data varied for each child. The average was 58 days (range 28-112 days).

Only within-subject air pollution effects were analyzed in this study. Between-subject effects were not reported. Each subject was analyzed individually.

Compared to other studies, there was a lot of baseline information provided for the children.

Possible bias due to misreporting of an asthma episode on a given day. Asthma symptom ascertainment was only based on the subjective reporting by each child without any clinical validation.

The individual pollutant exposures could have been misclassified in this study because a regional average from ambient monitors was substituted for individual exposures.

Potential cofounders and effect modifiers such as other outdoor pollutants, exposure to airborne pollens and moulds, other meteorological factors and respiratory infection were not considered.

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM

Author: Blanquart, C., Giuliani, I., Houcine, O., Jeulin, C., Guennou, C., Marano, F.	**Internal ID:** 463
Title: In Vitro Exposure of Rabbit Tracheal Epithelium to SO_2: Effects on Morphology and Ciliary Beating	

Year: 1995
Abstract: The aim of this in vitro study was to characterize the direct effects of short-term exposure to low concentrations of sulfur dioxide on both the morphology and the physiology of rabbit tracheal primary cultures. Scanning electron microscopy (SEM) studies revealed that ciliated cells exposed for 1 hr to 10ppm or 30 ppm SO_2 exhibited aggregated cilia. Transmission electron microscopy revealed numerous swollen mitochondria in cells exposed to 30 ppm SO_2 for 1 hour. This morphological damage to cells was coupled with physiological alterations. A 25% decrease in ciliary beta frequency (CBF) was measured in cells exposed to 30 ppm SO_2. This inhibition was partially reversible within 24 hr. This SO_2 concentration also induced a significant depletion of cellular ATP content, which was completely restored after a 24 hr recovery period. A correlation was found between cellular ATP level depletion and CBF decrease.
Objective: The purpose of the present work was to determine and to characterize the direct effects of a single 1hr exposure to SO_2, in a concentration range of 0-30ppm, on both the morphology and the ciliary activity of rabbit tracheal epithelial cells. To do this we developed a cell exposure chamber that allows the testing of gaseous pollutants in primary cell cultures.

Overall study design:

Exposure level	Exposure frequency/duration	Species	Strain/ Breed	Age	Sex	Number of animals	Pre-trial health	NOAEL	LOAEL
1, 10, 30ppm	1 hour	Rabbit	Fauve de Bourgogne	1 month	No info	No info	No info	n/a	n/a

Observations:

Cytotoxic Effects

There was a linear increase of sulfur IV species present in the culture medium after a single 1 hr exposure to increasing concentrations of SO_2. Viability was measured in 9-day-old cultures immediately after exposure of the cells to the gas. A significant decrease in cell viability could be observed after a 1hr exposure to SO_2 concentrations higher than 10ppm. The percentage of cytotoxicity measured after a 1hr exposure to 30 ppm SO_2 always remained lower than 30%, whereas about 40% of cells died when exposed to 50 ppm SO_2.

Morphological Studies

Significant ultrastructural alterations could be observed immediately after a 1hr exposure to 10ppm SO_2: ciliated cells of the explants and of the outgrowth exhibited aggregated cilia which were close together and flattened on the cell surface. SEM evaluation of the cell surface also revealed areas of individual cell loss immediately following SO_2 exposure. These alterations were acutely observed on cell cultures exposed to 30ppm SO_2 for 1 hour.

TEM revealed swollen mitochondria in cell cultures exposed to 30 ppm SO_2.

Ciliary Activity

Cell viability remained higher than 70% at exposures of 10-30ppm SO_2. Ciliary activity of ciliated cells exposed to 10-30ppm SO_2 for 1 hour was significantly inhibited. This inhibition increased proportionally with SO_2 concentration. Exposure to 30 ppm SO_2 for 1hr caused 25% inhibition of CBF. Beat frequencies of untreated cells on day 4 of culture were about 6.5Hz (baseline value) and decreased to 5Hz after a 1hr exposure to 30ppm SO_2. A partial recovery occurred within 24 hours for all cultures although beat frequencies always remained lower than the baseline value.

Cellular ATP content

SO_2 induced a depletion of ATP content in all treated cultures after a single 1hr exposure. Cellular ATP contents were significantly reduced only after a 30ppm exposure: the mean values decreased about 36%. Cellular ATP contents were also determined after a 24 hr recovery period, and were found to be restored: the ATP level of epithelial cells previously exposed to 10ppm SO$_2$ was not found to be significantly different from the intracellular ATP level of control cultures. On the other hand, cultures that had been exposed previously to 30 ppm SO$_2$ exhibited a cellular ATP content higher than control cultures: the cellular ATP level significantly increased by about 50% .

A correlation could be established between the percentage of cellular ATP depletion and CBF decrease following SO$_2$exposusre.

Author's Conclusions:

"Our results showed that the ciliary activity alteration of ciliated epithelial cells directly exposed to SO$_2$ was likely to have at least two causes; the aggregation of cilia and the cellular ATP depletion."

"In conclusion, our present work shows that a short-term exposure to sulfur dioxide caused both morphological and physiological alterations of the tracheal ciliated epithelium at low concentrations close to those observed to induce respiratory diseases in vivo. These alterations are a modification of the mitochondrial function and a reversible inhibition of ciliary activity. We also demonstrate that this inhibition is likely to be due, at least in part, to both the aggregation of cilia and the cellular ATP depletion."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	(+) Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+) **1 month** b) Age *Guideline: Adult, i.e.,8-12 weeks.* **(no info)**_ c) Sex **(no info)** d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* **(n/a in vitro study)** e) Weight variation *Guideline: Below 20% variation* **(no info)** f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(+) **except lot no.** Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided?

	(+) for some of the experiments 0, 10, 30ppm Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? (+) Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? **(n/a in vitro study) but a type of cell exposure chamber was used** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? **(+) gas monitored continuously** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(+) for cells** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(n/a in vitro study** Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* **(n/a in vitro study** Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* **(n/a in vitro study** Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet*

	(n/a in vitro study Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(+) for cells** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. **(-)** Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(n/a in vitro study** Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* **(n/a in vitro study** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(n/a in vitro study** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) (+) Was a control group assayed? (+) Is the control regime appropriate? **(n/a in vitro study** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	(+) Were appropriate endpoints examined? (+) Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? (+) Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **((n/a in vitro study** Was individual data reported for each test animal?
G. Data analysis:	(+) Are the statistical methods used appropriate?

	(+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (some) Are the observed trends statistically significant?
H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? **(more detail needed)** Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? **(no extrapolations made to humans)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Morphological damage to cells was observed along with physiological alterations. The findings showed that cells exposed to 10 or 30 ppm SO_2 exhibited aggregated cilia and exposure to 30 ppm $SO_2$for 1 hour resulted in swollen mitochondria. A decrease in ciliary beat was measure in cells exposed to 30ppm SO2 but this effect was partially reversible within 24 hrs. 30ppm SO_2exposure also induced a significant depletion of cellular ATP which was completely restored after a 24hr recovery period. The findings give some evidence to the mechanism of action of SO_2 toxicity. Study findings differ from other similar investigations, where SO_2-induced inhibition of airway ciliary motility was at least in part due to the impairment of cyclic AMP production.

Confidence index:

The study was generally well done with good detail in the methods. For the determination of sulfur in the cells, the control regime is not stated.

The authors briefly review the process of creating tracheal epithelium primary cultures, but do not mention how rabbits were housed, fed or watered. In addition, no mention is made of possible confounding factors such as weight, gender or previous health; however, this may have been due to the in vitro nature of the study. Chamber conditions for the cell cultures are described in detail. Explanation is given on the use of low exposure limits.

The number of cultures was not reported. Also, no raw data is given in the report.

Review – Confidence index ranking:

Moderate to high

CASE-CONTROL REVIEW FORM

Author: **Wong, C.M., Ma, S., Hedley, A.J., Lam T.H.** **Internal ID:** 464

Title: Effect of Air Pollution on Daily Mortali8ty in Hong Kong

Year: 2001

Abstract: In different weather conditions, constituents and concentrations of pollutants, personal exposure, and biologic responses to air pollution may vary. In this study we assessed the effects of four air pollutants on mortality in both cool and warm seasons in Hong Kong, a subtropical city. Daily counts of mortality, due to all nonaccidental causes, and cardiovascular and respiratory diseases were modeled with daily pollutant concentrations [24-hr means for nitrogen dioxide, sulfur dioxide, and particulate matter < 10 microm in aerodynamic diameter (PM_{10}); 8-hr mean for ozone]. using Poisson regression. We controlled for confounding factors by fitting the terms in models, in line with those recommended by the APHEA (Air Pollution and Health: a European Approach) protocol. Exposure-response relationships in warm and cool seasons were examined using generalized additive modeling. During the cool season, for a linear extrapolation of 10th-90th percentiles in the pollutant concentrations of all oxidant pollutants, NO_2, SO_2, and O_3, we found significant effects on all the mortality outcomes under study, with relative risks (RR) of 1.04-1.10 ($p < 0.038$, except $p = 0.079$ for SO_2 on respiratory mortality). We observed consistent positive exposure-response relationships during the cool season but not during the warm season. The effects of PM_{10} were marginally significant (RR = 1.06; $p = 0.054$) for respiratory mortality but not for the other outcomes ($p > 0.135$). In this subtropical city, local air quality objectives should take into account that air pollution has stronger health effects during the cool rather than warm season and that oxidant pollutants are more important indicators of health effects than particulates.

Objective: In this study we assessed the effects of air pollution on mortality outcomes and identified which pollutants would contribute most to the effect in Hong Kong, a subtropical city in the Asian Pacific rim.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
24 hour average: **Warm:** $18.3 \pm 13.0 \mu g/m^3$ (range 1.9-83.9$\mu g/m^3$) Cool: $17.2 \pm 11.6 \mu g/m^3$ (range 1.1-90.1$\mu g/m^3$)	Study period 1995-1997	Male and female	No info	N=mortality counts over 1096 days	No info	n/a	n/a

Observations:

Relative risk (RR) and 95% confidence interval of the best single lagged-day effects y linear extrapolation for a 10th – 90th percentile change in pollutant concentration (1995-1997): whole year

Causes of mortality	Lag day	RR(95% CI)(unadjusted)	p-value
Nonaccidental	1	1.03(1.02-1.05)	0.000
Cardiovascular	1	1.05 (1.02-1.08)	0.001
Respiratory	0	1.04(1.01-1.08)	0.010

(**NOTE: the mean 10th – 90th percentile change for the whole year is not given but for the warm season it was approximately 33μg/m^3 and for the cool season it was approximately 29.7μg/m^3)

During the warm season, there were no significant effects ($p>0.1$) for SO_2 for all mortality outcomes. During the cool season, without adjustment for copollutants the RRs at the best lagged days were significantly greater than unity in nonaccidental and cardiovascular mortality ($p<0.002$).

Relative risk and 95% confidence interval of best single lagged day effects by linear extrapolation for a 10th-90th percentile change in pollutant concentration (1995-1997) without adjustment for a copollutant

Cause of mortality	Warm Season		Cool season	
	RR(95% CI)	p-Vale	RR(95% CI)	p-value
Nonaccidental	1.02 (0.99-1.04)	0.170	1.04(1.02-1.07)	0.001
Cardiovascular	1.01(0.97-1.05)	0.546	1.07(1.02-1.11)	0.002
Respiratory	1.02(0.99-1.09)	0.101	1.04(1.00-1.09)	0.079

During the warm season, no exposure-response relationships were observed when SO_2 was <30μg/m^3, but there were some linear or nonlinear relationships above that concentration. During the cool season, positive exposure response relationships for concentrations of 0-40μg/m^3 SO_2 were observed.

During the cool season, for linear extrapolations of 10th-90th percentiles in the pollutant concentrations of all oxidant pollutants, NO_2, SO_2, and O_3, we found significant effects on all the mortality outcomes under study, with relative risks (RR) of1.04-1.10 ($p < 0.038$, except p= 0.079 for SO_2 on respiratory mortality). We observed consistent positive exposure-response relationships during the cool season but not during the warm season.

Author's Conclusions:

"In this subtropical city, local air quality objectives should take into account that air pollution has stronger health effects during the cool rather than warm season and that oxidant pollutants are more important indicators of health effects than particulates."

"For most of the pollutants, the effects were non-significant after adjusting for a copollutant; this may arise from a problem of multicollinearity, except for NO_2 in cardiovascular mortality and SO_2 in both nonaccidental and cardiovascular mortality. These observations together with strong positive exposure-response relationships for NO_2 and SO_2, suggest that NO_2 and SO_2 have independent effects and may be better indicators of effects on mortality in this subtropical city."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	. (+)Is the number of subjects sufficient to produce statistically significant results? . (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? . (Not enough info)Are the subjects a representative sample of the population to which extrapolations will be made? . (n/a no controls)Were identical 'criteria for exclusion' applied to both cases and controls? . (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	. (+) fixed monitorsWas the exposure concentration measured? . (+) Study period 1995-1997Is the exposure duration known?
C. Equipment:	. (-)Was a full description of the monitoring devices provided? . (no info)What was the sensitivity of the monitoring devices employed?
D. Procedural:	. (-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? . (n/a no controls)Were appropriate controls recruited? . (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) . (+)Was the timing and length of follow-up appropriate? . (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	. (n/a no controls)Was the assessment identical for cases and controls? . (+)Were appropriate endpoints examined? . (not enough info) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) . (+)Were accepted assessment methods employed? . (+)Were assessment measures sufficiently sensitive? . (+)Was the nature and severity of symptoms graded against an explicitly defined scale? . (+)Did qualified personnel conduct the exams? . (+)Was the timing and frequency of assessment logical? . (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? . (-)Was individual data reported for each subject? . (no withdrawals)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	. (+)Are the statistical methods used appropriate? . (+)Are confidence intervals reported? . (+)Did the investigator appropriately interpret the statistics?

	(some)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (-)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? (+)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (No)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The effects of SO_2 were significant for all of the mortality outcomes in the cool season but not in the warm season. However, the average levels of SO_2 for the cool and warm seasons were very similar, at 17μg/m^3 and 18μg/m^3 respectively. The greater variability of weather in the warm period and differences in activity in the two seasons may have prevented the actual exposure-response relationships between air pollution and mortality from being readily observable. These factors, in addition to the frequent use of air-conditioning would prevent the actual exposure response relationships between air pollution and mortality from being readily observable. The nonsignificant relative risk estimates for SO_2 on respiratory disease may be due to the small change in concentration from the warm season to the cool season. A positive exposure-response relationship for SO_2 and all the outcomes during the cool season was observed, but not for the warm season.

Depending on the location, different components of air pollution contribute more to health effects. This study attempted to determine which pollutants contribute most to the health effects in Hong Kong. Consequently, this report may contribute to the understanding of the differences in effects and mechanisms between Western and Eastern populations. Except for respiratory mortality, no strong effects of particulate pollutants were observed in HK, although the levels were high.

Confidence index:

The literature review was well done. There was very little information regarding the monitoring stations and sampling methods.

Comparison with other studies was pretty detailed with differences among studies stated. The authors made comparisons to other studies but did caution about extrapolating their results outside the Hong Kong area. The relative risk values were all very low. Data for this study were obtained from the Census and Statistics department, Hong Kong Observatory and the Environmental Protection Department. The authors utilized a variety of statistical techniques to limit possible confounders such as seasonality, temperature, and dummy variables for days of the week, holidays and influenza epidemics.

Exposure assessment is a weakness in this type of study.

Review – Confidence index ranking:

Moderate to low

CASE-CONTROL REVIEW FORM

Author: Alberdi Odriozola, J.C., Diaz Jimenez, J., Montero Rubio, J.C., Miron Perez, I.J., Pajares Ortiz, M.S., Ribera Rodrigues, P.	**Internal ID:** 465
Title: Air pollution and mortality in Madrid, Spain: a time-series analysis	
Year: 1998	

Abstract: OBJECTIVE: To assess the relationship, if any, between air pollutant (sulfur dioxide and total suspended particulate) levels and mortality in the city of Madrid during the period 1986-1992, controlling for weather, season, and influenza epidemics. METHODS: Daily death counts were obtained from the Regional Mortality Registry. Pollution data were supplied by the Municipal Monitoring Network. Time-series analysis methodology was used to assess the link between non-accidental as well as circulatory- and respiratory-disease mortality, on the one hand, and mean daily concentrations of SO_2 and total suspended particulate (TSP), on the other. Multivariate autoregressive integrated moving-average (ARIMA) models were used to adjust for season, temperature, relative humidity, and influenza. A sensitivity analysis was run to assess the robustness of the estimators. RESULTS: Graphical analysis revealed a linear relationship between mortality and TSP. The relationship was logarithmic in the case of SO_2. TSP lagged 1 day and SO_2 lagged 3 days with an independent effect on mortality. This relationship was produced without the detection of a minimal threshold in emission values. CONCLUSIONS: These results support the hypothesis of an association between pollution levels and mortality between 1986-1992 in Madrid. Additional measures designed to reduce pollution levels without compromising thermal comfort should be implemented.

Objective: To assess the relationship, if any, between air pollutant (sulfur dioxide and total suspended particulate) levels and mortality in the city of Madrid during the period 1986-1992, controlling for weather, season, and influenza epidemics.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Annual Station 1: 70.5±52.2μg/m^3 (4-479μg/m^3) Station 2: 78.8±44.7μg/m^3 (9-374μg/m^3) **Summer** Station 1: 39.9±18.6μg/m^3 (7-97μg/m^3) Station 2:	January 1986-December 1992	Male and female	All ages	Total number of deaths/day (all ages and both gender) (mean±SE): **Annual** Nonaccidental Causes: 60.3±11.3 Cardiovascular Causes:23.7±6.5 Respiratory Causes:5.8±3.1 **Summer** Nonaccidental:54.4±9.41 Cardiovascular:20.1±5.3 Respiratory: 4.7±2.4	No info	N/a	N/a

55±25.4μg/m^3 (9-153μg/m^3) Winter Station 1: 105.4±61.1μg/m^3 (13-479μg/m^3) Station 2: 108.8±48.1μg/m^3 (17-374μg/m^3)				**Winter** Nonaccidental:66.9±10.6 Cardiovascular:27.4±6.1 Respiratory: 7.0±3.4			

Observations:

For SO_2, annual nonaccidental deaths (all ages) reached significance at lag 3. When analyzed seasonally, the significance shifted to lag1 for summer and lag2 for winter. When analyzed by gender, the significance shifted again. For men, (annual=lag3, summer=lag2, winter=lag3). For the women (annual=lag3, summer=lag1). In the over-64-years age group the effect advanced to lag1 for both sexes and for all-cause mortality.

For SO_2, annual cardiovascular deaths (all ages) reached significance at lag 1. When analyzed seasonally, the significance was lag1 for summer. When analyzed by gender, the remained at lag1 for men for annual and summer. For the women (summer=lag3).

For SO_2, annual, summer, and winter respiratory deaths (all ages) reached significance at lag 1. When analyzed by gender, the significance association for men occurred at (annual=lag0 and summer=lag1). For the women, (annual=lag0 and winter lag1).

When temperature was controlled for, the SO_2 induced effect disappeared in both winter and summer.

Spectral analysis of TSP and SO_2 revealed the same deterministic structure for each of the four monitoring stations, namely, a downward trend and statistically significant annual, weekly, and 3-day periodicities. Mortality registered a rising trend as well as a yearly and a 6-monthly seasonal component but no weekly cycle. In the case of temperature there was a statistically significant annual cycle only. The relationship between nonaccidental deaths and TSP was linear, whereas that observed for SO_2 was logarithmic.

SO_2 exhibited different behaviour patterns, depending on sex, age, and season. On a seasonal basis the association between circulatory disease mortality and pollution was observed exclusively in summer, whereas that noted for respiratory diseases was in evidence throughout the year.

Inclusion of influenza periods in the model led to a 50% fall in the magnitude of the cold-temperature coefficients. Only TSP was related to mortality by cause, sex, and age.

Author's Conclusions:

"Although the SO_2/mortality relationship has not been clearly established, its logarithmic nature indicates an even more striking effect for low concentrations."

"These results support the hypothesis of an association between pollution levels and mortality between 1986-1992 in Madrid. Additional measures designed to reduce pollution levels without compromising thermal comfort should be implemented."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(Not enough information provided) Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls)Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitoring Was the exposure concentration measured? (+) 7-year study period Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (Not provided) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls)Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-)Are the statistical methods used appropriate? (some)Are confidence intervals reported? (Not enough data provided) Did the investigator appropriately interpret the statistics? (some). Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel?

	. (+)Was the design of the study appropriate to investigate the primary objective? . (+)Were assumptions reasonable? . (-)Are the methods reproducible? . (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? . (+)Do the conclusions make biological sense? . (-)Are the extrapolations reasonable? . (+)Is the paper in a peer-reviewed journal? . (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The authors report a logarithmic relationship in the case of SO_2 and mortality as indicated by graphical analysis. A log relationship may indicate the nonexistence of a threshold. However, this analysis is not clearly presented in the study. There are significant relationships reported between SO_2 and mortality for different lag times; however, the lag values depend on how the data are analysed.

Findings from this study indicated that the rise in respiratory-disease mortality was primarily due TSP. An association between TSP, SO_2 and cardiovascular mortality was then established.

Confidence index:

The most significant problem with this study is the lack of data. For example, the results simply state the lag times at which the associations with SO_2 and mortality are statistically significant. However, no RR, OR, correlation values, p-values or confidence intervals are presented. No nonsignificant relationships are shown; only significant data are presented. In the table presenting the significant lag results for mortality and SO_2, some data are missing and there is no explanation. For example, for women, there may be no value in the table for SO_2 levels and mortality from cardiovascular diseases in the winter. Is this because the analysis wasn't done or is it because the associations were not significant?

The SO_2 levels were measured at two stations. It is not clear whether the data were combined or if the data at each station were analysed separately. In Figure 1b, correlations between daily organic deaths and SO_2 are shown for Station 1 only.

The Tables and Figures need much more detail in the title, description, definition of variables etc.

Much of the discussion of results is very vague with further explanation needed. For example, it is stated that SO_2 showed a log relationship with mortality, but there are little data and no graphical analysis to support this. When the data were stratified by season, the months of September, October, April and May were not included in either summer or winter. There is the possibility of misclassification bias by reporting the cause of death as 'cardiovascular' when it should be 'respiratory'. Statements are made about significantly stronger associations when the data are stratified by age. A reference is made to Table 3; however, these data are not presented. Table 3 presents the data stratified by season and gender only. The total deaths per age group (under 65;over 65) are shown in Table 2, but significance not given in Table 3. All deaths of residents occurring outside the Madrid city limits were excluded. There are no details on the number of deaths that may have been excluded from the analysis. It is not clear why the significant lags would change when looked at data seasonally, or by gender etc. The discussion is very vague in this area. This study utilized data from the Regional Mortality Registry, which did not provide previous health history or demographic data, items that could confound findings. The number of monitoring stations was inadequate to cover the subject area involved.

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM – IN VITRO - HUMANS

Author: Riechelmann, H., Kienast, K., Schellenberg, J., Mann, W.J.	**Internal ID:** 466
Title: An in vitro model to study effects of airborne pollutants on human ciliary activity	
Year: 1994	
Abstract: A method to study the effects of airborne pollutants on ciliary activity of isolated human respiratory cells is presented. Human respiratory cells were scraped from nasal cavities of 25 healthy volunteers and washed in Ringer's solution. The cells were placed on polycarbonate membranes (3 microns pore size) and kept in macroplate holders swimming on Ringer's solution. Cells were thus kept humid and were supplied with nutrients through the pores of the membrane by capillary forces, while their surface was exposed to the gaseous environment. Isolated respiratory cells were exposed to SO_2, NO_2, and mixtures of SO_2 and NO_2 in various concentrations for 30 min and for 2 h. Exposure to synthetic air served as control. Ciliary beat frequency was measured using video-interference contrast microscopy, before and after exposure to the various gases. Exposure of isolated respiratory cells to a non-toxic gaseous environment resulted in a 20% reduction of ciliary beat frequency. A concentration-dependent decrease of ciliary beat frequency following exposure to SO_2 in concentrations ranging between 2.5 and 12.5 ppm was found. Exposure to NO_2 up to 2 h in concentrations ranging between 3 ppm and 15 ppm did not decrease ciliary beat frequency. No cumulative effect was found, if a mixture of SO_2 (2.5 ppm) and NO_2 (12 ppm) was applied.	
Objective: An in vitro model to investigate acute effects of volatile substances on ciliary activity of human respirator cells is presented. The basic principle is to expose isolated human respiratory cells to a toxic atmosphere. Using various concentrations of SO_2 and NO_2 the reproducibility and concentration-response performance in this experimental set up was evaluated.	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of humans**	**Pre-trial health**	**NOAEL**	**LOAEL**
2.5, 5, 7.5, 10, and 12.5 ppm	30 min and 120 min	Human	n/a	24±4 y	11 female 14 male	Cells from 25 humans Number of cells varied per experiment	Non smoker	n/a	n/a

Observations:

Ciliary beat frequency revealed a concentration – dependent reduction ($p<0.001$) following exposure to SO_2($n=14$) from 7.8±0.8 Hz (control) to 4.7±1.7 Hz (2.5ppm), 2.8±1.4 Hz (5ppm), 1.5±1.4 Hz (7.5ppm), 0.7±1.2Hz (10ppm) and 0.28±0.75Hz (12.5ppm) respectively. The effects of SO_2 were in part reversible, when the samples were suspended in phosphate-buffered solution following SO_2 exposure ($n=8$). Exposure of isolated respiratory cells to a non-toxic gaseous environment results in a 20% reduction of ciliary beat frequency.

Author's Conclusions:

"In conclusion, gas exposure of isolated human respiratory cells is a cost effective and time saving method to evaluate possible risks of volatile substances for ciliated respiratory cells. This method offers interesting perspectives in detecting occupational risk factors for the respiratory tract while working with volatile substances. Exposure to 2.5 ppm SO_2 for 30 minutes resulted in a significant decrease of ciliary activity."

"Comparing ciliary beat frequency before and after exposure to synthetic air for 30-120 min, a reduction of approximately 20-25% was consistently found. The effects of SO_2 on ciliary activity were in part reversible, when the cells were suspended in phosphate-buffered solution following gas exposure. This suggests that the effect of SO_2 on ciliary activity are in part due to pH-related cellular dysfunction."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

****Human In Vitro Study** so many of the guidelines below are not applicable.**

A. Test animals:	(n/a) Is the number of animals sufficient to produce statistically significant results? └ *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie └ *Guideline: Rat, or justification for use of another mammalian specie.* (+) b) Age └ *Guideline: Adult, i.e.,8-12 weeks.* (+) c) Sex (+) d) Pre-test health └ *Guideline: Females should be nulliparous and non-pregnant.* (no info) e) Weight variation └ *Guideline: Below 20% variation* (not stated) f) Source └ *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(+) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided? (+) Was there a gradient of exposure levels? └ *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective?

	(-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(not stated)** Were both nominal and actual exposure concentrations recorded? **(+)** Was the duration of exposure appropriate to investigate the primary objective? **(+)** Was the duration of exposure precisely defined? **(n/a)** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* **(+)** Was the mode of administration appropriate? **(+)** Was the frequency of administration appropriate to investigate the primary objective? **(-)** Was evidence provided to justify the dosing regime? (not stated) Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	**(n/a)** Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* **(n/a)** Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* **(n/a)** Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* **(n/a)** Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* **(n/a)** Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*
D. Equipment:	**(+)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. **(-)** Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?

E. Procedural:	**(n/a)** Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* **(n/a)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(n/a)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(n/a)** Was a control group assayed? **(+)** Is the control regime appropriate? **(n/a)** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	**(+)** Were appropriate endpoints examined? **(+)** Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(+)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(+)** Was the timing and frequency of assessment logical? **(-)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each test animal?
G. Data analysis:	**(+)** Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(+)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
H. Interpretations:	**(+)** Was the original objective addressed? **(-)** Are the results novel? **(+)** Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable?

	(-) Are the methods reproducible? **(no details)** Did either the study design or the statistical analysis limit confounding variables? **(+)** Do the conclusions make biological sense? **(+)** Are the extrapolations reasonable? **(+)** Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

This study suggests that the effects of air exposure on ciliary beat frequency are uniform and predictable. A concentration-dependent decrease of ciliary beat frequency following exposure to SO_2 in concentrations ranging between 2.5 and 12.5 ppm was found. The study used human respiratory cells. The results of this study may provide important evidence as to the hazardous effects on the human mucociliary transport system.

This study used a cost-effective method of studying the ciliary beat frequency of respiration cells. In addition, this study exposed human respiratory cells to a water-saturated gaseous environment unlike many previous studies in which respiratory cells were sensitive to dehydration.

Confidence index:

The literature review is inadequate.

The methods are described very briefly. The gas exposure system was diagrammed and environmental conditions were detailed.

There was some consistency reported in studies with guinea pigs.

There was no justification given for the SO_2concentrations and exposure times used nor was there any mention of previous studies to find the appropriate dosage levels. A gradient of exposure levels were used and a control was employed.

This study does not limit many confounding factors, such as age.

Treatment of the cultures was well defined in the report. Confidence intervals were not given in the statistical analysis.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Roemer, W., Hoek, G., Brunekreef, B., Haluszka, J., Kalandidi, A., Pekkanen, J.	**Internal ID:** 467

Title: Daily variations in air pollution and respiratory health in a multicentre study: the PEACE project

Year: 1998

Abstract: The Pollution Effects on Asthmatic Children in Europe (PEACE) study is a multicentre study of the acute effects of particles with a 50% cut-off aerodynamic diameter of 10 microm (PM_{10}), black smoke (BS), sulphur dioxide (SO_2) and nitrogen dioxide (NO_2) on the respiratory health of children with chronic respiratory symptoms. The study was conducted in the winter of 1993/1994 by 14 research centres in Europe. A total of 2,010 children, divided over 28 panels in urban and suburban locations, was followed for at least 2 months. Exposure to air pollution was monitored on a daily basis. Health status was monitored by daily peak expiratory flow (PEF) measurements and a symptom diary. The association between respiratory health and air pollution levels was calculated with time series analysis. Combined effect estimates of air pollution on PEF or the daily prevalence of respiratory symptoms and bronchodilator use were calculated from the panel-specific effect estimates. Fixed effect models were used and, in cases of heterogeneity, random effect models. No clear associations between PM_{10}, BS, SO_2 or NO_2 and morning PEF, evening PEF, prevalence of respiratory symptoms or bronchodilator use could be detected. Only previous day PM_{10} was negatively associated with evening PEF, but only in locations where BS was high compared to PM_{10} concentrations. There were no consistent differences in effect estimates between subgroups based on urban versus suburban, geographical location or mean levels of PM_{10}, BS, SO_2 and NO_2. The lack of association could not be attributed to a lack of statistical power, low levels of exposure or incorrect trend specifications. In conclusion, the PEACE project did not show effects of particles with a 50% cut-off aerodynamic diameter of 10 µm, black smoke, sulphur dioxide or nitrogen dioxide on morning or evening peak expiratory flow or the daily prevalence of respiratory symptoms and bronchodilator use.

Objective: In this paper, the effect estimates on respiratory health for the separate panels are used to calculate combined effect estimates for various air pollutants using meta-analysis techniques. In addition to combining effect estimates of all locations, differences between locations based on geographic location, urban/suburban location and composition of air pollution mixture were also investigated.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Urban areas: 2.7-113.9µg/m^3 Suburban areas: 3.4-64.9µg/m^3	Study period: Winter 1993/1994 (at least 2 months)	No info	6-12y	This study is a combined analysis of 14 studies. Each study contained an urban group and a suburban group. The following range is the range of subjects per study. Urban: n=50-91 Suburban: n=60-84 Total number of subjects: 2,010	Chronic respiratory symptoms	**n/a**	**n/a**

Observations:

No clear associations between PM_{10}, BS, SO_2, or NO_2 and morning PEF, evening PEF, prevalence of respiratory symptoms or bronchodilator use could be detected.

Most combined odds ratios were below 1.00, with significant OR for SO_2 lag0 on cough prevalence and SO_2 lag2 on the prevalence of upper respiratory symptoms.

There were no consistent differences in effect estimates between subgroups based on urban versus suburban, geographical location or mean levels of PM_{10}, BS, SO_2 and NO_2. The lack of association could not be attributed to a lack of statistical power, low levels of exposure or incorrect trend specifications.

*Statistically significant

Author's Conclusions:

"In conclusion, the Pollution Effects on Asthmatic Children in Europe (PEACE) project did not show clear effects of particles with a 50% cut-off aerodynamic diameter of 10μm, black smoke, sulphur dioxide or nitrogen dioxide on morning or evening peak expiratory flow or the prevalence of respiratory symptoms and bronchodilator use."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (+) **collected but not included in paper** Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (+)**assuming suburban subjects are controls** Were identical 'criteria for exclusion' applied to both cases and controls? (+) **No information given on incentive** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **Fixed monitors** Was the exposure concentration measured? (+)**study period at least 2 months per panels** the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? **(No info provided)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) **Details provided in a previous study** Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (+)**assuming suburban group is control** Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)

E. Data collection:	(+)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+)Were assessment measures sufficiently sensitive? (+)Was the nature and severity of symptoms graded against an explicitly defined scale? (-)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? **(some)**Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (+/-) **Withdrawals not listed, but explanations for exclusions given** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? **(some)**Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (-)**More detail given in other PEACE studies** Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(No biologically relevant conclusions)**Do the conclusions make biological sense? **(No extrapolations made)**Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There was no clear association between SO_2 and respiratory health. Odds ratios were typically below 1.00, which means that an increase in air pollution was associated with a decrease in prevalence, which was opposite to the expected association. The authors state that these unclear associations or in some cases lack of associations are not due to a lack of statistical power, low levels of exposure or incorrect trend specifications.

The combination of the panel-specific estimates into an aggregate estimate did not show a clear effect of air pollution on either PEF or the daily prevalence of respiratory symptoms or bronchodilator use. The PEACE project did not show effects of SO_2 on morning or evening peak expiratory flow or the daily prevalence of respiratory symptoms and bronchodilator use.

Confidence index:

This study was not able to show any clear association between SO_2 and respiratory health effects; however, the authors do an excellent job of identifying faults in the study design and possible reasons for the lack of associations. All significant and nonsignificant results are also provided so the reader can draw her own conclusions.

Stronger associations may have been identified if smaller susceptible subgroups were analyzed. This is provided in another PEACE study.

Children were selected by a parent-completed screening questionnaire which could have resulted in selection bias. There is no indication of any blinding.

There was extensive baseline information collected for each child, but it is detailed in another study.

There was considerable heterogeneity between the panel results. Heterogeneity was present for all effect estimates of all components on all symptoms and bronchodilator use, but most consistently for 7 day mean effect estimates. Authors suggest this is due to linguistic and cultural differences in respiratory symptoms reporting and medication use. The differences are unlikely to be caused by differences in study design, methods or data analysis, according to the authors, because all centres used the same protocol for fieldwork and at analysis.

The authors justify methods with previous studies.

The reaction of a panel to air pollution might be influenced by panel composition. For example, children who were selected only on the basis of a positive answer to the nightly cough question had a lower prevalence of lower respiratory symptoms, upper respiratory symptoms and phlegm than children selected on asthma symptoms and reacted differently to air pollution in some of the panels. This may affect the relationship between the regression slopes and indicators of air pollution composition.

Suburban SO_2 data was missing for two of the panels.

Respiratory infections may have influenced the children's lung function.

It was also suggested that the timing of the morning PEF measurement may have been a problem. The measurements were taken just after getting up, which means that a long period of indoor exposure preceded the measurement. Exposure misclassification may be an issue since exposure measurements were taken from ambient monitoring stations.

The few associations that were significant could have been due to chance to the large amount of statistical testing.

It was difficult to fully characterize the children involved, given the large sample size. The authors attempted to limit confounding factors by choosing centres that had no large industrial sources, no major traffic emissions and were sufficient in size to select enough subjects.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Tanaka, H., Honma, S., Nishi, M Igarashi, T., Teramoto, S., Nishio, F., Abe, S.	**Internal ID:** 468

Title: Acid fog and hospital visits for asthma: an epidemiological study

Year: 1998

Abstract: The aim of this study was to elucidate the adverse respiratory effects of naturally occurring acid fog. In total, 102 adult asthmatic patients (44 nonatopic and 58 atopic) were studied for a 2 yr period (January 1992 to December 1993) in Kushiro, a city with only a small industrial area, located in the northern-most island in Japan. Fog occurred on 378 out of 731 days, and the acidity of the fog ranged from pH 3.32 to 6.91 (mean pH 4.95). The association between hospital visits for asthma and meteorological factors or air pollutants was investigated. In nonatopic patients, fog, high ozone and water vapour pressure, low day-to-day temperature differences, low concentrations of atmospheric NO and NO_2 contributed significantly ($p<0.05$) to increasing hospital visits. In atopic subjects, fog, high water vapour pressure, low levels of atmospheric NO_2 and SO_2 contributed significantly to hospital visits ($p<0.05$). In Poisson regression analysis the remaining factors of significance ($p<0.01$) for nonatopic asthma were fog and low NO and for atopic asthma were high water vapour pressure and low SO_2 ($p<0.05$). A weak but significant correlation was observed between the number of hospital visits and the mean pH of the foggy day ($r=-0.38$, $p<0.05$) in nonatopic asthmatic patients, not in atopic asthma. On foggy days, gaseous air pollutant levels were significantly ($p<0.01$) lower than on fog-free days. It was concluded that, naturally occurring acid fog may have a weak bronchoconstrictive effect which appears to be more influential in nonatopic asthmatic subjects than in atopic subjects.

Objective: The purpose of this study was to elucidate the respiratory effects of naturally occurring acid fog on adult nonatopic and atopic asthmatics. The results of the association between ambient acid fog and daily hospital visits are reported, including hospitalization, emergency room and outpatient visits for asthma symptoms obtained by medical records, and compared with the effects of other meteorological variables and gaseous air pollutants in Kushiro city during a 2yr period from 1992 to 1993.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Foggy Day (n=378d): 3.2±2.4ppb Fog Free Day (n=353d): 3.7±1.9ppb	2 year study period	Nonatopic:12 male and 32 female Atopic: 33 male and 25 female	Nonatopic: 52±16 y (21-79y) Atopic: 41±16y (15-70y)	Nonatopic: 44 Atopic: 58	Asthmatic	n/a	n/a

Observations:

Most of the results refer to the effects of the acid fog as a whole and not to the separate pollutants. The acid fog contains multiple stimuli that may have an adverse effect on human airways. Only the observations referring to SO_2 specifically are included here.

SO_2 was significantly lower on the foggy days than on the fog free days ($p<0.01$)
The odds ratios of hospital visits and 95% confidence intervals for SO_2 and meteorological variables

	Nonatopic patients		**Atopic patients**	
	OR	95%CI	OR	95%CI
SO_2 ≥5ppb versus < 5 ppb	1.18	0.96-1.46	0.78	0.66-0.93

In nonatopic patients, fog, high ozone and water vapour pressure, low day to day temperature differences, low concentrations of atmospheric NO and NO_2 contributed significantly to increasing hospital visits. In atopic subjects, fog, high water vapor pressure, low levels of atmospheric NO_2 and SO_2 contributed significantly to hospital visits. In Poisson regression analysis the remaining factors of significance for nonatopic asthma were fog and low NO and for atopic asthma were high water vapour pressure and low SO_2. A weak but significant correlation was observed between the number of hospital visits and the mean pH of the foggy day in nonatopic asthmatic patients, not in atopic asthma. On foggy days, gaseous air pollutant levels were significantly lower than on fog-free days.

Author's Conclusions:

"In conclusion, hospital visits for asthma exacerbation in nonatopic asthmatic patients were found to increase on foggy days, especially on days with lower pH fog. Because levels of gaseous air pollutants on foggy days were lower than those on fog-free days, air pollutants may have been absorbed by the fog and the acidity of fog water may then be increased. Naturally occurring acid fog may have a weak bronchoconstrictive effect, which appears to be more influential in nonatopic asthmatic patients than in atopic subjects."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(Not enough info provided) Is the number of subjects sufficient to produce statistically significant results? (+)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls)Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitoring station Was the exposure concentration measured? (+) 2 year study period Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (-)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval?

	(n/a no controls) Were appropriate controls recruited? **(-)**Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) **(+)**Was the timing and length of follow-up appropriate? **(+)**Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)**Was the assessment identical for cases and controls? **(Not enough information provided)** Were appropriate endpoints examined? **(-)**Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(Not enough info provided)** Were accepted assessment methods employed? **(Not enough info provided)** Were assessment measures sufficiently sensitive? **(-)**Was the nature and severity of symptoms graded against an explicitly defined scale? **(+)**Did qualified personnel conduct the exams? **(+)**Was the timing and frequency of assessment logical? **(-)**Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)**Was individual data reported for each subject? **(-)**Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	**(+)**Are the statistical methods used appropriate? **(+)**Are confidence intervals reported? **(+)**Did the investigator appropriately interpret the statistics? **(-)**Are the observed trends statistically significant?
G. Interpretations:	**(+)**Was the original objective addressed? **(-)**Are the results novel? **(+)**Was the design of the study appropriate to investigate the primary objective? **(+)**Were assumptions reasonable? **(-)**Are the methods reproducible? **(+) just meteorological variables** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(+)**Do the conclusions make biological sense? **(-)**Are the extrapolations reasonable? **(+)**Is the paper in a peer-reviewed journal? **(-)**Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The purpose of the study was to address the respiratory effects of naturally occurring acid fog on adult nonatopic and atopic asthmatics. There was very limited information on the effects of gaseous SO_2 as this was not the specific objective of the study. This study did not find a statistically significant association between respiratory effects and SO_2 levels in atopic and nonatopic patients. It appeared that hospital visits by atopic subjects increased on low SO_2 days, which is inconsistent with most other studies. The level of SO_2 was significantly lower on foggy days than fog free days. However, the SO_2 concentration in the fog was not measured.

Results suggest that the inhalation of naturally occurring acid fog might have adverse respiratory effects, especially on nonatopic asthma. There is a trend towards an increase in hospital visits for asthma during periods of fog. Authors conclude that naturally occurring acid fog may have a weak bronchoconstrictive effect which appears to be more influential in nonatopic asthmatic subjects than in atopic subjects.

Confidence index:

The objective was to address the effects of acid fog, not specifically gaseous SO_2.

SO_2 concentrations were measured only in the gaseous phase and it is not know what the concentration of SO_2 in the fog water is.

More detail is needed on the statistical analysis.

The level of asthma severity differed considerably within the two groups.

Medical records were obtained for 102 of a possible 182 subjects. There is no indication as to whether this was done randomly.

Only subjects who visited a certain hospital were chosen. This group may not be representative of all asthmatics in this area.

Many of the patients received medication throughout the study, which may have made them less susceptible to asthmatic effects.

There were significant differences between atopic and nonatopic subjects in gender and age.

Individual hospital visits were noted retrospectively from the patients' medical records. However, total visits are not given. If the same patient visited 2 or more consecutive days, this was considered to be one visit. Any visit within 2 weeks of an acute exacerbation was not counted. The results may change depending on how visits were counted.

The results section of the study is very brief.

The authors provide information on the recruitment process and describes how subjects were categorized into nonatopic and atopic groups. To obtain information on personal exposure to fog, subjects completed questionnaires, answering only "yes" or "no" to limit variation in responses. Possible confounding factors such as meteorological variables were controlled for at the observatory where air pollution data was also recorded.

A limitation of this study is that the use of outdoor fog-monitoring data to estimate personal exposure introduces error into the exposure assessment.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Hajat, S., Haines, A., Goubet, S.A., Atkinson, R.W., Anderson, H.R.	**Internal ID:** 469

Title: Association of air pollution with daily GP consultations for asthma and other lower respiratory conditions in London

Year: 1999

Abstract: BACKGROUND: Very few published studies have looked at the effects of air pollution on health in the primary care setting. As part of a large study to examine the association between air pollution and a number of health outcomes, the relationship between daily GP consultations for asthma and other lower respiratory diseases (LRD) and air pollution in London was investigated. METHODS: Time-series analysis of daily numbers of GP consultations controlling for time trends, seasonal factors, day of week cycles, influenza, weather, pollen levels, and serial correlation was performed. Consultation data were available from between 268 718 and 295 740 registered patients from 45-47 London practices contributing to the General Practice Research Database during 1992-4. RESULTS: Positive associations, weakly significant and consistent across lags, were observed between asthma consultations and nitrogen dioxide (NO_2) and carbon monoxide (CO) in children and particulate matter of less than 10 microm in diameter (PM_{10}) in adults, and between other LRD consultations and sulphur dioxide (SO_2) in children. A consistently negative association with ozone in children was observed in both disease categories. The effect estimates of most pollutants were much larger when analysed separately by season, particularly in the children: percentage change in asthma consultations during the warm season (April-September) for a 10-90th percentile increase in 24 hour NO2 lagged by one day = 13.2% (95% CI 5.6 to 21.3), with CO = 11.4% (95% CI 3.3 to 20.0), and with SO_2 = 9.0% (95% CI 2.2 to 16.2). In adults the only association consistent over different lag periods was with PM10 = 9.2% (3.7 to 15.1). The associations of pollution and consultations for LRD were increased mainly in the winter months: percentage change in consultations by children in winter with NO_2 = 7.2% (95% CI 2.8 to 11.6), CO = 6.2% (95% CI 2.3 to 10.2), and SO_2 = 5.8% (95% CI 1.6 to 10.2). CONCLUSIONS: There are associations between air pollution and daily consultations for asthma and other lower respiratory disease in London. The most significant associations were observed in children and the most important pollutants were NO_2, CO, and SO_2. In adults the only consistent association was with PM_{10}.

Objective: As part of a larger study in London which also analysed air pollution in relation to mortality, admissions, and accident and emergency consultations, we carries out a time series analysis of daily general practitioner consultations in London over he period 1992-1994.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
April-Sept.- 20.5±6.5μg/m^3 Oct-March: 22.0±9.0μg/m^3	Study period: January 1992-December 1994	Male and female	Children 0-14y Adults – 15-64 Elderly (65+)	Mean number of patients consulting each day for asthma were 14.0(9.8), 17.8(11.8), and 3.6(3.0) n the young, adult and elderly respectively, and there were 39.7(29.2), 73.8(51.1) and 41.4(27.4) patients consulting for other lower respiratory diseases.	No info	n/a	n/a

Observations:

The most significant associations were observed in children. Associations for adults and the elderly appeared to be independent of SO_2 concentrations.

Summary of results for SO_2 analysis of consultations for asthma. Percentage change in number of consultations for a 10-90th percentile change (approximately 18μg/m^3) in SO_2 presented at its most significant single/cumulative lag

Age Group	Single day lag	%change in number of consultations (95% CI)	p value	Cumulative Measure	% change in consultations (95%CI)	p value
All ages	2	3.6 (0.3-6.9)	0.03	0 to 2	4.4 (0.9-7.9)	0.01
0-14	1	4.9 (0.1-9.8)	0.04			

Summary of results for SO_2 analysis of consultations for lower respiratory disease (LRD) excluding asthma. Percentage change in number of consultations for a 10-90th percentile change (approximately 18μg/m^3) in SO_2 presented at its most significant single/cumulative lag

Age Group	Single day lag	%change in number of consultations (95% CI)	p value	Cumulative Measure	% change in consultations (95%CI)	p value
All ages	2	1.8(0.2-3.4)	0.03	0 to 2	2.2(0.4-4.1)	0.02
0-14	2	4.5(1.4-7.8)	0.004	0 to 3	5.7(1.7-9.7)	0.004

Results for analysis by season of lagged single day measurements. Percentage change in number of consultations for a 10-90th centile change ((approximately 15μg/m^3 for warm season and 20 μg/m^3 for the cool season) in SO_2 presented at its most significant single lag for all-year, warm and cool seasons.

Asthma		**All-year**		**Warm Season**		**Cool Season**	
Age Group	Lag (days)	%change in risk (95% CI)	p value	%change in risk (95% CI)	pvalue	% change in risk (95%CI)	p value
0-14	1	4.9 (0.1-9.8)	0.04	9.0(2.2-16.2)	0.009	2.0(-4.5-8.9)	0.5

LRD		**All-year**		**Warm Season**		**Cool Season**	
Age Group	Lag (days)	%change in risk (95% CI)	p value	%change in risk (95% CI)	pvalue	% change in risk (95%CI)	p value
0-14	2	4.5(1.4-7.8)	0.004	2.4(-2.6-7.7)	0.4	5.8(1.6-10.2)	0.006

Positive associations, weakly significant and consistent across lags, were observed between asthma consultations and nitrogen dioxide and carbon monoxide in children and particulate matter of less than 10 um in diameter in adults, and between other LRD consultations and sulfur dioxide in children .

The effect estimates of most pollutants were much larger when analyzed separately by season, particularly in the children: percentage change in asthma consultations during the warm season for a 10-90th percentile increase in 24 hour NO_2 lagged by one day = 13.2% with CO = 11.4% and with SO_2 = 9.0% (95% CI 2.2 to 16.2). In adults the only association consistent over different lag periods was with PM_{10} = 9.2%.

The associations of pollution and consultations for LRD were increased mainly in the winter months: percentage change in consultations by children in winter with NO_2 = 7.2%, CO = 6.2% and SO_2 = 5.8% (95% CI 1.6 to 10.2).

Single pollutant analysis for asthma and SO_2 in each age group showed statistical significance with percentage increases in the region of about 4%. There were positive associations with SO_2 and PM_{10}, although these were not statistically significant at the 1% level. The bubble plots for the LRD model in children showed a consistent exposure-response relationship in the model residuals with increases in NO_2 and SO_2 and also, with the exception of a few outliers, with PM_{10}.

Author's Conclusions:

"There are associations between air pollution and daily consultations for asthma and other lower respiratory diseases in London. The most significant associations were observed in children and the most important pollutants were NO_2, CO, and SO_2. In adults, the only consistent association was with PM_{10}."

"The results of this paper are likely to be underestimates for the following reasons: no distinction could be made between acute or follow up consultations; the use of monitoring sites for pollution levels does not allow for measurement of individual exposure; and the LRD group is a very heterogeneous disease group which could dilute effect estimates. Despite these limitations, the findings are consistent with the acute effects of air pollution on other health outcomes and show that primary care is a feasible source of data for showing effects of air pollution on health."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls)Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Fixed monitoring sitesWas the exposure concentration measured? (+) 3year study period Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (No information provided) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls)Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom)

	(+)Were accepted assessment methods employed? (-)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (+/-) some Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Most of the significant SO_2 findings in this study concerned GP consultations with children. For consultations for other LRD the bubble plots for SO_2 suggested a dose response relationship (weak) and SO_2 was statistically significant associated with an increase in consultations at two-day lags in children. This lag relationship may suggest an optimum time of two days for any detrimental effect of pollution on LRD in children to manifest itself and present to the GP. The fact that there were no significant findings in the adult and elderly groups may be due to shortcomings in the study design discussed below.

This study used a number of statistical tools were used to determine long term trends, seasonality and day of week fluctuations. Spectral analysis, and Poisson regression was further used to determine the effects of confounding factors. The paper's author notes that the results in the paper may be underestimates since the use of monitoring sites for pollution levels dies not allow for measurement of individual exposures.

Confidence index:

The introduction was very brief and the literature review was inadequate.

Average concentrations between SO_2 concentrations for the warm season and cool season are similar (only a 1.5μg/m^3 difference). This may be due to how the months were grouped.

SO_2 concentrations were obtained from 5 sites, but there is no information on the location of the sites or reliability of the data. The study does not make mention of the location of the monitoring stations involved, but directs readers to a separate review of UK air quality. The location of the stations would contribute to the validity of the data collected, depending on proximity to subjects involved and to industrial processes.

Some confounders were considered in the analysis.

Confidence intervals were wide.

Data was not reported for all time lags. For example, for 0-14years, association between asthma consultations and SO_2, only lag 1 was used. The lag used was simply reported as being the most significant lag.

The association between asthma and SO_2 in children decreased in the cool months. Increased use of medication in the winter months may be the reason for this. There may be different pollutant mixtures between seasons.

The seasonal analysis of LRD (excluding asthma) revealed an opposing pattern to that observed with asthma, with all significant associations occurring in the cool months. The difference in this diagnostic group could be due to the much higher number of consultations for these types of diseases in the winter months, leading to greater statistical power.

The LRD group is a very heterogeneous disease group, which could dilute effect estimates.

No distinction could be made between acute or follow-up consultations.

This study is modeled after the APHEA studies.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Harre, E.S.M., Price, P.D., Ayrey, R.B., Toop, L.J., Martin, I.R., Town, G.I.	**Internal ID:** 470
Title: Respiratory effects of air pollution in chronic obstructive pulmonary disease: a three month prospective study	
Year: 1997	
Abstract: BACKGROUND: A study was undertaken to investigate the relationship between air pollution levels and respiratory symptoms and peak expiratory flow rate (PEFR) in subjects with chronic obstructive pulmonary disease (COPD) living in Christchurch, New Zealand. METHODS: Forty subjects aged over 55 years with COPD completed twice daily diaries for three months during the winter of 1994. Subjects recorded respiratory symptoms, PEFR, outdoor activity, visits to doctor or hospital, and medication use. All were resident within a 5 km radius of the regional council's air pollution monitoring site. Daily and hourly mean pollutant levels (particulates (PM_{10}, nitrogen dioxide (NO_2), sulphur dioxide (SO_2) and carbon monoxide (CO)) were measured at the monitoring site. RESULTS: Pollution levels were generally low relative to those recorded in previous years. The New Zealand Ministry for the Environment guidelines for PM_{10} were exceeded on five occasions, and for CO six times. No association was found between PEFR and any of the pollution variables. A rise in the PM_{10} concentration equivalent to the interquartile range was associated with an increase in night time chest symptoms (relative risk 1.38, 95% CI 1.07 to 1.78). A rise in NO_2 concentrations equivalent to the interquartile range was associated with increased reliever inhaler use (relative risk 1.42, 95% CI 1.13 to 1.79) and for 24-hour lag analysis with increased nebuliser use (relative risk 2.81, 95% CI 1.81 to 4.39). There was no increase in the relative risk of other symptoms in relation to pollution levels. CONCLUSIONS: These effects, demonstrated in a small susceptible group of subjects with COPD, indicate that adverse outcomes can be measured in response to pollution levels that are within current guidelines.	
Objective: The specific objective was to establish whether air pollution levels currently observed in Christchurch during the winter months have any significant effects on pulmonary function, symptoms, and medication use in subjects with chronic obstructive pulmonary disease (COPD).	

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Range: 0-40μg/m^3 (approximate values from graph)	3 month study period	16 female 24 male	Mean 72 y Range 55-83y	40	Chronic obstructive pulmonary disease	n/a	n/a

Observations:

SO_2 levels were well within the WHO guidelines.
There was no significant association between any individual pollutant and either morning or evening PEFR. (For a 4.58μg/m^3 increase in SO_2 there was a 0.05% change in Morning PEFR (95%CI -0.24 to 0.36)and a -0.06 % change (95%CI -0.23 to 0.11)in Evening PEFR.)
There was a weak association between increased SO_2 levels and eye irritation. The relative risk was 1.17 (95% CI 0.99-1.38) of experiencing eye irritation in association with a mean increase in SO_2 level equivalent to the interquartile range of 4.58 μg/m^3.

The associations between SO_2 and nebuliser and reliever inhaler use were not significant. (Relative risk = 1.00 for nebuliser use and 1.05 for inhaler use.
There was no significant association between sulfur dioxide concentrations and health effects experienced by COPD patients.
Pollution levels were generally low relative to those recorded in previous years.

Author's Conclusions:

"These effects, demonstrated in a small susceptible group of subjects with COPD, indicate that adverse outcomes can be measured in response to pollution levels that are within current guidelines."

"We did not find any effect of SO_2 on chest symptoms or PEFR, although a raised SO_2 concentration was associated with eye irritation. Raised particulate (PM_{10}) concentrations were associated with increased night time chest symptoms. Higgins et al. did not measure PM_{10} levels in their study but speculate as to whether their observation that raised levels of SO_2 were associated with increased wheezing might reflect an effect of particulates which tend to covary with SO_2."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(-)Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)**Were identical 'criteria for exclusion' applied to both cases and controls? (-)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **Fixed monitoring site**Was the exposure concentration measured? (+) **three month study period** Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? **(No information provided)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)**Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (-)Were assessment measures sufficiently sensitive?

	(-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? **(no extrapolatiosn)**Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no significant findings with respect to SO_2 exposure. There was only a very weak association between SO_2 and eye irritation The small study population was self-selected and the magnitude of the effects seen was generally small. Thus, the results cannot be generalized to either the population as a whole or to all those with chronic obstructive pulmonary disease.

Confidence index:

There are problems with the subject selection for this study. The study subjects were self-selected which likely introduced bias. The results cannot likely be generalized to the whole population.

This study detailed the recruiting process and described baseline demographic data, medication use and lung function. In the beginning there were 61 subjects recruited from three different sources: (1) general practitioners in the central Christchurch area (n=25); (2) a community-based COPD support group (n=28) and (3) newspaper advertisements (n=8). Of the 61 subjects, 40 subjects were accepted for the study. However, it is stated that 19 were recruited from the newspaper advertisements (but the original pool was only n=8??).

There was, however, a power calculation estimating that the models have power greater than 95% to detect a 10% change in mean PEFR with a sample size of 40.

There was an extensive description of the statistical methods used; however, the discussion of the results was quite brief.

Subjects received individual flow meters and study diary with which to record details of respiratory symptoms. Throughout the study, diaries were reviewed and further training given to ensure completeness of data. Training effects were not considered even though some subjects had to be retrained during the study on how to use the peak flow meter and how to complete the diaries.

Subjects resided within 5 km of the monitoring station. Subjects were asked to omit recordings if traveling out of the study area for more than 24 hours. There are no details provided on number of absences.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Schwartz, J.	**Internal ID:** 471

Title: Short term fluctuations in air pollution and hospital admissions of the elderly for respiratory disease

Year: 1995

Abstract: BACKGROUND--Several recent studies have reported associations between short term changes in air pollution and respiratory hospital admissions. This relationship was examined in two cities with substantially different levels of sulphur dioxide (SO_2) but similar levels of airborne particles in an attempt to separate the effects of the two pollutants. Significant differences in weather between the two cities allowed the evaluation of that potential confounder also. METHODS--Daily counts of admissions to all hospitals for respiratory disease (ICD 9 460-519) were constructed for persons aged 65 years and older in two cities - New Haven, Connecticut and Tacoma, Washington. Each city was analyzed separately. Average daily concentrations of SO_2, inhalable particles (PM_{10}), and ozone were computed from all monitors in each city, and daily average temperature and humidity were obtained from the US weather service. Daily respiratory admission counts were regressed on temperature, humidity, day of the week indicators, and air pollution. A 19 day weighted moving regression filter was used to remove all seasonal and subseasonal patterns from the data. Possible U-shaped dependence of admissions on temperature was dealt with using indicator variables for eight categories each of temperature and humidity. Each pollutant was first examined individually and then multiple pollutant models were fitted. RESULTS--All three pollutants were associated with respiratory hospital admissions of the elderly. The PM_{10} associations were little changed by control for either ozone or SO_2. The ozone association was likewise independent of the other pollutants. The SO_2 association was substantially attenuated by control for ozone in both cities, and by control for PM_{10} in Tacoma. The magnitude of the effect was small (relative risk 1.06 in New Haven and 1.10 in Tacoma for a 50 $\mu g/m^3$ increase in PM_{10}, for example) but, given the ubiquitous exposure, this has some public health significance. CONCLUSIONS--Air pollution concentrations within current guidelines were associated with increased respiratory hospital admissions of the elderly. The strongest evidence for an independent association was for PM_{10}, followed by ozone. These results are consistent with other studies and suggest that lowering air pollution concentrations would have some impact on public health.

Objective: This study examined the association between air pollution and respiratory hospital admissions in two cities – New Haven, Connecticut and Tacoma, Washington.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
New Haven – Mean SO_2 29.8ppb Tacoma-Mean SO_2 16.8 ppb	Study period – Jan 1, 1988 – December 31, 1990	Male and female	65y or older	Mean Admissions/day: New Haven- 8.8 Tacoma-4.2	No info	**n/a**	**n/a**

Observations:

(Only considering observations made on SO_2 alone, not when compared with other pollutants)

SO_2 levels two days before were significant predictors of hospital admissions for respiratory disease.

New Haven

Winter (10.3 admissions/day Average seasonal SO_2 141 $\mu g/m^3$)
Spring (8.4 admissions/day Average seasonal SO_2 63 $\mu g/m^3$)
Summer (6.5 admissions/day Average seasonal SO_2 45 $\mu g/m^3$)
Autumn (7.1 admissions/day Average seasonal SO_2 63 $\mu g/m^3$)

Relative risk of respiratory hospital admissions and 95% confidence intervals for a 50 $\mu g/m^3$ increase in SO_2 in New Haven (concentrations taken two days before admission)
RR=1.03 95% CI=1-1.13 $p<0.05$

Tacoma

Winter (5.7 admissions/day Average seasonal SO_2 58 $\mu g/m^3$)
Spring (4.6 admissions/day Average seasonal SO_2 35 $\mu g/m^3$)
Summer (3.2 admissions/day Average seasonal SO_2 34 $\mu g/m^3$)
Autumn (3.4 admissions/day Average seasonal SO_2 50 $\mu g/m^3$)

Relative risk of respiratory hospital admissions and 95% confidence intervals for a 50 $\mu g/m^3$ increase in SO_2 in New Haven (concentrations from same day of admission)
RR=1.06 95% CI = 1.01-1.12 $p>0.02$

The SO_2 association was substantially attenuated by control for ozone in both cities, and by control for PM_{10} in Tacoma. In two pollutant models, SO_2 was no longer significant after controlling for PM_{10} was controlled for.

*Statistically significant

Author's Conclusions:

"Air pollution concentrations within current guidelines were associated with increased respiratory hospital admissions of the elderly. The strongest evidence for an independent association was for PM_{10}, followed by ozone. These results are consistent with other studies and suggest that lowering air pollution concentrations would have some impact on public health."

"The situation with SO_2 is more mixed. Studies in Europe consistently report associations with SO_2, but the correlation between SO_2 and airborne particles is high in all of these studies. In studies where the correlation between SO_2 and airborne particles was lower, weaker or no associations were seen with SO_2. In locations with low concentrations of SO_2, the associations seen with airborne particles were similar to those seen in Europe. All this suggests that the observed SO_2 associations are confounded by airborne particles. Further examination of locations with low correlations between the two pollutants or with very low concentrations of one pollutant, will be needed to confirm or refute this conclusion."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (Not enough info provided)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls)Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+)Fixed monitoring sites Was the exposure concentration measured? (-)Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (no information)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls)Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (Not enough information provided)Were assessment measures sufficiently sensitive? (n/a)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (+)Was individual data reported for each subject? (no withdrawals)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? (some)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed?

	(-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Choosing two locations where the ratio of SO_2 to airborne particles differs considerably and comparisons of findings between such locations may provide greater insight into which pollutant is the primary correlate with adverse health outcomes. However, the climate between the two locations differed substantially and may have affected the results. The association between SO_2 and admissions was also inconsistent between the two locations. For New Haven, SO_2 levels two days before were significant predictors of hospital admissions of respiratory disease. However, in Tacoma, it was the value on the same day rather than two days before that was predictive of hospital admissions. In both cities control for ozone substantially weakened the association with SO_2. Results The relative risk values were all low.

This study suggests that air pollution may be responsible for causing perhaps 5% of the respiratory hospital admissions of the elderly. The author suggests that, while it may not be possible to entirely remove air pollution, a 20% reduction in air pollutants could contribute to a 1% reduction in hospital admissions.

Confidence index:

Authors attempted to maximize between-area exposure variations to represent different extremes of exposure distribution.

Exposure was measured by fixed monitoring sight. A full description of the monitoring devices was not given nor is the sensitivity of the monitoring devices known.

There is no background information or baseline data provided for the subjects. Subjects were all over 65 years old.

It is not clear whether the mean SO_2 concentrations reported for each season are seasonal averages or daily averages.

The relative risks were close to 1.

The two locations also differed in climate, which may have confounded the analyses. Tacoma had a more moderate and less variable climate than New Haven. The study attempted to correct for this possible confounding factor.

For New Haven, it was the concentration of SO_2 two days before that was associated with hospital admissions, whereas for Tacoma, it was the same day concentration that was of concern.

Weaknesses in this type of study are in exposure assessment.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Peters, A., Dockery, D.W. Heinrich, J., Wichmann, H.E.

Internal ID: 472

Title: Short-term effects of particulate air pollution on respiratory morbidity in asthmatic children

Year: 1997

Abstract: Short-term effects of air pollution (consisting primarily of sulphur dioxide and particulate matter but with low acidity) on respiratory morbidity were studied in asthmatic children from Sokolov, Czech Republic. Eighty nine children with asthma, who recorded daily peak expiratory flow measurements, symptoms and medication use in a diary, were analysed for 7 months during the winter of 1991-1992. Air pollution measurements included: SO_2, total suspended particulates (TSPs), inhalable particles, ie. particulate matter of aerodynamic diameter < or = 10 µm, particle strong acidity (PSA) and fine particle sulphate concentration (SO_4). Linear and logistic regression analyses estimated the impact of air pollutants adjusted for trend, temperature and weekend and autocorrelated errors. Exposure to elevated levels of air pollution was associated with decreased peak expiratory flow rates, increased respiratory symptoms, increased prevalence of school absence and fever, and increased medication use. Prolonged exposure to particle SO_4 showed the largest effect estimates. At the end of January, an air pollution episode occurred, during which respiratory symptoms, prevalence of fever, school absence and asthma medication increased. The association between respiratory symptoms and particulate SO_4 was highly dependent on this episode, whilst the associations between lung function and SO_4 as well as between fever and SO_4 were still observed when this air pollution episode was excluded. Some evidence was found that exposure to air pollution might have enhanced the respiratory symptoms while children were experiencing respiratory infections. In this study, a panel of children with mild asthma experienced small decreases in peak expiratory flow and increased dyspnoea in association with fine particles formed during air pollution episodes.

Objective: Short-term effects of air pollution (consisting primarily of sulphur dioxide and particulate matter but with low acidity) on respiratory morbidity were studied in asthmatic children from Sokolov, Czech Republic.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Mean - 71µg/m^3 Max - 383µg/m^3	Study period – Winter 1991-1992 (7months)	Male and female	6-14 years	89	Asthmatic (76 medically diagnosed asthma)	n/a	n/a

Observations:

There was a statistically significant decrease in the change in peak expiratory flow (PEF) in the evening -1.67(-2.76 to -0.58) L/min for a 65µg/m^3 increase (5-day mean) in SO_2.

Odds Ratios for respiratory symptoms associated with an increase in air pollutants by one interquartile range (defined as 67 µg/m^3 for current day and 65 µg/m^3 for the five day mean of SO_2) adjusted for trend, temperature and weekend (versus weekdays) with 95% confidence intervals in parentheses:
There were statistically significant associations between the five-day mean of SO_2 and phlegm (OR=1.16 (1.11 to 1.21)) and runny nose (OR=1.07 (1.04-1.10)).

Odds Ratios for prevalence of fever, school absence and medication use associated with an increase in air pollutants by one interquartile range (defined as 67 $\mu g/m^3$ for Current day and 65 $\mu g/m^3$ for the five day mean of SO_2) adjusted for trend, temperature and weekend (versus weekdays) with 95% confidence intervals in parentheses:
There were statistically significant associations between the current day value of SO_2 and fever (OR=1.16 (1.02-1.32)) and between five-day means of SO_2 and fever (OR=1.52 (1.29-1.80)) and school absence (OR=1.10 (1.05-1.15)) and increased use of β-agonist (OR=1.32 (1.16-1.50)).

NOTE: The SO_2 levels were very variable throughout the study period. Episodes of elevated air pollution for more than 7 days occurred repeatedly during the winter 1991-1992. A mean SO_2 concentration of 71 $\mu g/m^3$ (max 383 $\mu g/m^3$) was measured during the period of annals. Much lower air pollution concentrations were recorded during the 5-week period between mid-December and mid-January. A second distinct air pollution episode occurred at the end of January 1992 when SO_2 concentrations were above 200 $\mu g/m^3$ for 12 days.

The prevalence of fever increased with elevated levels of SO_2 and SO_4 on the same day, and was even more pronounced with 5-day means of SO_2, SO_4, and PSA. School absence showed no association with exposure to air pollution on the same day, but was predicted by prolonged exposure to SO_2 and SO_4. The use of β-agonists was associated with concurrent exposure to TSP and prolonged exposure to all winter-type pollutants. The children increased their maintenance asthma medication in association with 5-day means of all air pollutants. Prevalence of symptoms was reduced at higher temperatures for cough, phlegm and dyspnoea, whilst the symptoms runny nose, fever, school absence and use of asthma medication did not show an effect of temperature. The strong association between fever and SO_2 and SO_4 and PSA could raise the suspicion that an influenza epidemic might have coincided with the air pollution episode at the end of January.

*Statistically significant

Author's Conclusions:

"Asthmatic children from Sokolov, Czech Republic, showed a small, statistically significant association between lung function and winter air pollution."
"Prevalence of the respiratory symptoms increased in association with concurrent and prolonged exposure to SO_4, showing stronger associations for the 5 day means than the same day. Other pollutants, such as SO_2 and PSA, showed similar or weaker effects. Respiratory symptoms assess different aspects of the respiratory health in children. Phlegm and runny nose are symptoms of the upper respiratory tract, whilst cough and dyspnoea are symptoms of the lower airways. The data presented here suggested that both the upper and lower airways were affected by air pollution. Other indicators for increased morbidity, such as the prevalence of school absence, fever and the use of asthma medication, showed associations with elevated levels of SO_2 and SO_4. Both beta-agonist and theophylline use appeared to be related to increased exposure to air pollution."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(Not enough information provided)** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? **(More information needed)**Was the method of recruitment appropriate? Would the participation incentive employed cause the selection

	of an unrepresentative sample population?
B. Exposure conditions:	(+) **(fixed monitoring site)**Was the exposure concentration measured? **(Study period – 7 months)**Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? **(No information provided)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) **Reference included for paper detailing exposure assessment** Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)**Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)**Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+/-)Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+) **medical asthma diagnosis** Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported? (+)Did the investigator appropriately interpret the statistics? **(some)**Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable?

	(-)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? (**No extrapolations made**)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were some weakly significant associations between SO_2 levels and respiratory symptoms in the children. However, the symptoms examined could have been caused by variables other than air pollution. For example the association between SO_2 and fever could raise the suspicion that an influenza epidemic might have coincided with the air pollution episode at the end of January. Also, over half of the children used asthma medication, which may reduce their susceptibility and produce weaker associations. The changes in PEF values and symptom prevalence did appear to follow the general pattern of fluctuations in SO_2 levels. Perhaps the study lacked the statistical power to produce more strongly significant results.

Confidence index:

Only one monitoring site was used to obtain SO_2 concentrations in central Sokolov. The residences of the children varied from 5km to 25km from the monitoring station. Exposure assessment is a weakness of this study design.

The subjects were chosen from a larger panel study. There is no indication as to whether the subjects were chosen randomly.

There were some differences in the children. Out of the 89 children, 76 had doctor confirmed asthma, but only 46 of the children were taking medication during the winter period under investigation.

The authors report 89 children with asthma were studied, but also state that 97 children were enrolled. There is no information on the 8 children missing from the analyses.

The diary data appeared more detailed than other studies. For example, symptoms at rest and during exertion were recorded. However, parents completed the diaries and may have been influenced by knowledge of air pollution episodes that winter.

PEF measurements were checked for validity and regarded as invalid if the values were the same for more than 14 days. However, fourteen days seems quite lenient. Previous studies discard readings if values are the same for 3 or more days.

Several confounding variables were addressed.

The odds ratios for the significant associations were very low.

Confidence intervals are reported. The conclusions in this study do further support the conclusions of previous studies.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Xu, X., Dockery, D.W., Christiani, D.C., Li, B., Huang, H. | **Internal ID:** 474

Title: Association of Air Pollution with Hospital Outpatient Visits in Beijing

Year: 1995

Abstract: Data collected at a community-based hospital in Beijing, China, were analyzed in an assessment of the association of air pollution with daily outpatient visits. Total suspended particle (TSP) measurements were available for 210 d (mean, 388 µg/m^3; maximum, 1,255 µg/m^3), and sulfur dioxide (SO_2) measurements were available for 2 d (mean, 119 µgrams/m^3; maximum, 478 µg/m^3). The average number of daily hospital outpatient visits was 1,386; approximately 8.5% of these visits were to the surgery department, 7.9% were to the pediatrics department, and 20.6% were to the internal medicine department. A large increase in nonsurgery outpatient visits was observed in association with increases in both SO_2 and TSP in linear regression models, after adjusting for temperature, humidity, season, and day of the week. The estimated effects (in which the most polluted days were compared with the least polluted days) on nonsurgery outpatient visits were increases of 20% (SE = 5%) and 17% (SE = 4%) in association with increases in SO_2 and TSP, respectively. In a department-specific analysis, the association was found to be 1.5- to 2.0-fold stronger for pediatrics and internal medicine visits than for other types of visits. The separate associations of SO_2 and TSP with internal medicine visits remained statistically significant when both SO_2 and TSP were considered simultaneously and when adjustment was made for surgery visits. SO_2 and TSP were found to be significant, independent predictors of internal medicine visits in both winter and summer. The association between outpatient visits and air pollution appeared to be stronger in summer than in winter, although the summer daily mean SO_2 concentrations was only 17 µg/m^3 (maximum, 51 µg/m^3). These data, together with data obtained in previous studies, provide coherent evidence that current air-pollution levels in Beijing are associated with adverse health outcomes, and they also suggest that air pollution in Beijing needs to be controlled more effectively.

Objective: In this study, daily outpatient visits at Fuxing Hospital, a community-based hospital in Beijing, were used to investigate the acute effects of air pollution.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean - 119 µg/m^3 Max – 478 µg/m^3	Study period – 355 days	Male and female	No info	Mean number of hospital outpatient visits: 1386/day	No info	n/a	n/a

Observations:

*Observations concerning the effects of the interdependence of SO_2 and TSP were not included in this review.

Locally weighted regression analysis, including temperature, humidity, day of the week and season, showed a negative association between daily surgery visits and SO_2 and showed a positive associate between daily nonsurgery visits and SO_2.

SO_2 was a highly significant predictor for total and nonsurgery outpatient visits. The estimated effects (in which the most polluted days were compared with the least polluted days) on nonsurgery outpatient visits were increases of 20% (Standard error = 5%). **(pvalues and confidence intervals not given)** In a department-specific analysis, the association was found to be 1.5- to 2.0 fold stronger for pediatrics and internal medicine visits than for other types of visits. No significant association of air pollution with surgery visits was observed. **(pvalues and confidence intervals not given)**

In a season specific regression analysis, with adjustments made for temperature, humidity, day of the week, and surgery visits, SO_2 was found to be significant predictor of internal medicine visits in both summer and winter. The associate between outpatient visits and air pollution appeared stronger in summer than in winter, although the mean daily SO_2 concentration was only 17 $\mu g/m^3$ (maximum, 51 $\mu g/m^3$)

Author's Conclusions:

"These data, together with data obtained in previous studies, provide coherent evidence that current air-pollution levels in Beijing are associated with adverse health outcomes, and they also suggest that air pollution in Beijing needs to be controlled more effective."

"Given that TSP and SO_2 may be highly correlated with other pollutants emitted from the same source, we cannot rule out the possibility that TSP and SO_2 are simply surrogates for a complex mixture of air pollutants. A more accurate way of assessing the effects of separate pollutants would be to use human chamber studies, which have been used to evaluate the acute effects of short-term exposure. In addition, the actual composition, the physical state, and the size distribution of particulates may profoundly affect their toxic action…better assessment of exposure is needed in future studies."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls)Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(Fixed monitoring sites)Was the exposure concentration measured? (+) 355 day study period Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (No info provided) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls)Were appropriate controls recruited? (-)Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate?

	(+)Was the nature of follow-up appropriate? (*i.e.,* questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a no controls)Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.,* information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (Not enough info provided) Were accepted assessment methods employed? (Not enough info provided Were assessment measures sufficiently sensitive? (-)Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? (-)Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-)Are the statistical methods used appropriate? (-)Are confidence intervals reported? (-)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (-)Were assumptions reasonable? (-)Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.,* additional chemical exposure, etc.)? (+)Do the conclusions make biological sense? (-)Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (-)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The study states that SO_2 and TSP were found to be significant, independent predictors of internal medicine visits in both winter and summer. The association between outpatient visits and air pollution appeared to be stronger in summer than in winter, although the summer daily mean SO_2 concentration was quite low. The authors claim that their findings (along with findings from previous studies) provide coherent evidence that current air pollution levels in Beijing area are associated with adverse health outcomes, including decreased pulmonary function, respiratory symptoms, increased numbers of hospital visits and increased mortality. However, no details from the previous studies are given which makes it difficult to see the consistency with the present study. Also, because many patients do not visit doctors in local clinics or hospitals, outpatient records from a hospital may not reliably reflect the true morbidity in a given community. That results presented in this study are not strong enough evidence for the above conclusions. This study reiterates the need for better exposure measurements and identifying the effects of the separate pollutants.

Confidence index:

There is an error in the abstract. SO_2 measurements were actually measured for 206 days, not 2 days.

However, daily air samples were collected for only 2-3 weeks per month of the study period, resulting in a potentially significant amount of missing data. 206 days of data were collected out of a total 355 days.

There is an error in Table 1. Surgery visits are reported as mean = 1386 surgery visits per day, while actually this value represents the mean number of total hospital visits per day. Surgery visits represent only 8.5% of this value.

Table 2 was described as follows:

Table 2: **Linear Regression Analysis of Estimated Effects (Percentage Increase) of SO_2 and TSP on Hospital Outpatient Visits: Comparison between Most Polluted and Least Polluted Days.** When the results were described in the body of the paper they were also referred to as % increase. However, the columns in the tables were given the units $\mu g/m^3$ in error, it is assumed. Table 3 contained the same error.

A statistically significant relationship is reported to exist between increased levels of $SO_2$and increased number of daily outpatient visits; however, p-values and confidence intervals are not reported.

Patients in China are required to pay a registration fee for a hospital visit. Therefore, study population only includes patients who can afford or are willing to pay the fee and may not be representative of the general population.

A major limitation of this study was the lack of specific diagnostic information within department visits.

Exposure assessment is also a limitation.

The concentrations of SO_2 varied considerably throughout the study period.

Consistency reported with previous studies; however, no details are provided to justify the consistency.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Hernandez-Garduno, E., Perez-Neria, J., Paccagnella, A.M., Pina-Garcia, M.A., Munguia-Castro, M., Catalan-Vazquez, M., Rojas-Ramos, M.	**Internal ID:** 475

Title: Air Pollution and Respiratory Health in Mexico City

Year: 1997

Abstract: To determine the effect of air pollution in Mexico City on respiratory health, patient visits for upper respiratory tract infections were monitored in five clinics. Levels of ozone, nitrogen dioxide, carbon monoxide, and sulfur dioxide, and climatological variables were collected. Correlations of filtered data revealed an association between NO_2 and O_3 with an increase in visits to clinics because of respiratory problems. Autoregressive analysis indicated that pollutant levels/respiratory visits associations remained significant even after simultaneous inclusion of temperature, suggesting that air pollution was associated with 10 to 16% of the clinic visits. The relative risk indicated that high levels of O_3 and NO_2 could increase the total number of clinic visits to between 19 and 43% above average. The other pollutants and the control group did not demonstrate significant associations.

Objective: We decided to conduct this study to determine the association between air pollutants and respiratory visits to the five clinics of the ISSEMYM (Instituto de Seguridad Social del Estado de Mexico y Municipios) located in the northern part of Mexico City.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Not given!?!	Study period: 1 year	No info	No info	Approx. 28, 471 patients	No info	n/a	n/a

Observations:

The only observation for $SO_2$was that the Pearson correlation coefficient between SO_2 levels and respiratory visits was negative. The only two pollutants that were associated with total respiratory visits were the maximum same-day levels of NO_2 and O_3. There was no mention of SO_2 past the introduction.

*Statistically significant

Author's Conclusions:

No specific conclusions re $SO_2$and respiratory visits.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	. (+) Is the number of subjects sufficient to produce statistically significant results? . (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study?

	(more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (+) Were identical 'criteria for exclusion' applied to both cases and controls? (+) Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **but data not provided** Was the exposure concentration measured? (+) **study period – 1 year** Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(more info needed)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (-)Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a) Was the assessment identical for cases and controls? **(more info needed)** Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	**(limited statistical analysis)** Are the statistical methods used appropriate? (-) Are confidence intervals reported? **(no interpretation)** Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate tne primary objective?

	(more info needed) Were assumptions reasonable? **(-)** Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(no conclusions re SO_2)** Do the conclusions make biological sense? **(no extrapolations re SO_2)** Are the extrapolations reasonable? **(+)** Is the paper in a peer-reviewed journal? **(-)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

SO_2 was negatively correlated with respiratory visits. Therefore no further analysis was done. There was no attempt at an explanation for the negative correlation.

Findings suggest that SO_2 does not play a role in respiratory health. However, the author suggests that future similar studies be conducted to contribute more baseline information in the future.

Confidence index:

There is very limited information on the monitoring.

Only 2 monitoring stations were used. Exposure misclassification is likely a limitation.

The levels of $SO_2$are not provided in the paper.

Only able to collect health data from 5 medical units.

Extensive prefiltering of data before correlation analysis.

Limited information was reported regarding factors such as socioeconomic information or health history.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Salinas, M., Vega, J.	**Internal ID:** 476
Title: The effect of outdoor air pollution on mortality risk: an ecological study from Santiago, Chile	
Year: 1995	
Abstract: The aim of this ecological study was to investigate the effect of outdoor air pollution on the mortality risk of metropolitan inhabitants in Santiago de Chile. Cause-specific deaths by the day for the years 1988-1991 in Santiago de Chile were extracted from mortality data tapes of the National Center for Statistics. Deaths from accidents were excluded. Total and some specific respiratory diseases deaths were compared calculating the risk of death by municipality and month of the year using age-adjusted standardized mortality ratios (SMRs) controlling for socioeconomic level. Daly counts of deaths were regressed using a Poisson model on the total and fine suspended particles, SO_2, CO and ozone on the preceding day, controlling for temperature and humidity. A clear pattern in the geographical distribution risk of death, both for general mortality and specific respiratory causes (pneumonia, COPD and asthma) was found using SMR, with higher values in the most polluted areas regardless of socioeconomic and living conditions. A highly significant positive association was found between total mortality and both fine suspended particles and CO level. The association remained significant for those days with fine suspended particles level below 150 μg/dl suggesting a no-threshold effect for the total number of deaths. These results are in agreement with previously reported associations, and they add to the body of evidence showing that particulate pollution is associated with increases in daily mortality.	
Objective: The purpose of the present study was to determine the effect of air pollution on daily mortality in Santiago and to analyse whether the geographical distribution of the risk of death within the Metropolitan Area of Santiago was due to the bad quality of urban air.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Not provided!	1988-1991	Male and female	No info	Approximate population per municipality 41000-328900	No info	n/a	n/a

Observations:

There were no results given for the effects of SO_2 exposure on mortality risk. The authors do state that SO_2 was measured but no data on SO_2 levels is given. Therefore, the Review-Assessment below was not completed.
*Statistically significant

Author's Conclusions:

There were no specific conclusions for SO_2 effects. The conclusions mention particulate pollution only.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from

guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	✓ Is the number of subjects sufficient to produce statistically significant results? ✓ Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? ✓ Are the subjects a representative sample of the population to which extrapolations will be made? ✓ Were i ✓ Was th d cause the selection of an unrepresenta
B. Exposure conditions:	✓ Was th ✓ Is the e
C. Equipment:	✓ Was a full description of the monitoring devices provided? ✓ What was the sensitivity of the monitoring devices employed?
D. Procedural:	✓ Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? ✓ Were appropriate controls recruited? ✓ Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) ✓ Was the timing and length of follow-up appropriate? ✓ Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	✓ Was the assessment identical for cases and controls? ✓ Were appropriate endpoints examined? ✓ Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) ✓ Were accepted assessment methods employed? ✓ Were assessment measures sufficiently sensitive? ✓ Was the nature and severity of symptoms graded against an explicitly defined scale? ✓ Did qualified personnel conduct the exams? ✓ Was the timing and frequency of assessment logical? ✓ Was raw data provided to permit the reader to arrive at his/her own conclusions? ✓ Was individual data reported for each subject? ✓ Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	✓ Are the statistical methods used appropriate? ✓ Are confidence intervals reported? ✓ Did the investigator appropriately interpret the statistics? ✓ Are the ob

No SO_2 information or analysis provided in this study.

No SO_2 information or analysis provided in this study.

G. Interpretations:	✓ Was the original objective addressed? ✓ Are the results novel? ✓ Was the design of the study appropriate to investigate the primary objective? ✓ Were assumptions reasonable? ✓ Are the methods reproducible? ✓ Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? ✓ Do the conclusions make biological sense? ✓ Are the extrapolations reasonable? ✓ Is the paper in a peer-reviewed journal? ✓ Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:
There were no findings for SO_2 effects on mortality risk in Santiago Chile.
Confidence index:
No SO_2 data or analysis.
Very inadequate literature review.
The introduction did not talk about specific pollutants.

Review – Confidence index ranking:

Low

NON-CLINICAL REVIEW FORM

Author: Farone, A., Huang, S., Paulauskis, J., Kobzik, L.	**Internal ID:** 477

Title: Airway Neutrophilia and Chemokine mRNA Expression in Sulfur Dioxide-Induced Bronchitis

Year: 1995

Abstract: Airway inflammation in acute and chronic bronchitis includes a prominent neutrophil influx. Using a rat model of sulfur dioxide (SO_2)-induced bronchitis, we investigated the role of the polymorphonuclear leukocyte (PMN) chemokines macrophage inflammatory protein-2 (MIP-2) and KC. Adult female rats were exposed to 230 ppm SO_2 for 5 h/day for periods of 1 day to 5 wk. Immunohistochemical identification of rat PMNs in trachea cryostat sections allowed quantitation of a marked neutrophil influx into airways of bronchitic rats (PMNs/trachea ring = 55 +/- 26.2 [1 day SO_2] versus 3.6 +/- 2.7 [air]; n = 5, P < or = 0.05). Northern analysis of trachea homogenates demonstrated induction of KC and MIP-2 mRNA expression after 1 day of SO_2 and persistence of increased expression after longer exposure periods examined. Pretreatment of rats with dexamethasone (0.5 mg/kg) prior to a 1-day acute SO_2 exposure prevented induction of chemokine mRNA and abrogated neutrophil influx completely (PMNs/trachea ring = 6.6 +/- 8.8 versus air controls; n = 5, P = 0.96). To determine if chemokine inhibition by dexamethasone could be further studied in vitro, the rat alveolar macrophage cell line NR8383 was treated with dexamethasone (10(-7) M) before stimulation with lipopolysaccharide (10 micrograms/ml). Pretreatment with dexamethasone substantially decreased induction of both MIP-2 and KC mRNA in response to lipopolysaccharide, indicating the potential utility of in vitro systems to identify additional anti-inflammatory agents. These studies support the hypothesis that the chemokines MIP-2 and KC mediate airway neutrophil influx in both acute and chronic SO_2-induced bronchitis in the rat.

Objective: In this study, we characterized levels of neutrophil influx and chemokine mRNA in the tracheas of rats with SO_2 induced chronic bronchitis.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Species**	**Strain/ Breed**	**Age**	**Sex**	**Number of animals**	**Pre-trial health**	**NOAEL**	**LOAEL**
230ppm	Varied: 5h/day for 1day, 2 days, 1, 2, 4, or 5 wks	Rats	CD	Adult	Female	5 animals per treatment group	No info	n/a	n/a

Observations:

Neutrophil Influx during Acute Bronchitis

Substantially increased numbers of PMNs were present in tracheas of animals after acute (1day) exposure to SO_2 compared with minimal numbers of PMNs seen in the tracheas of control animals exposed to filtered air. Quantitation of PMNs confirmed the qualitative visual impression, indicating a statistically significant increase in neutrophils with 1-day SO_2 exposure (55±26.2 PMNs per tracheal ring) compared with control (3.6±2.7 PMNs) ($n=5$, $p<0.05$).

Chemokine mRNA Expression during Acute Bronchitis

Expression of chemokine mRNA was markedly induced after acute 1day exposure to SO_2 and this induction was decreased by dexamethasone pretreatment.

Chemokine mRNA Expression during Chronic Bronchitis

Substantial increases in KC and MIP-2 mRNA expression within tracheal samples were seen after exposures of 1,2 and 4 weeks compared with animals exposed to control filtered air.

Chemokine mRNA Expression In Vitro

Results from these studies show a LPS mediated induction of both MIP-2 and KC mRNA that was significantly inhibited by pretreatment with dexamethasone.

Author's Conclusions:

"These studies support the hypothesis that the chemokines MIP-2 and KC mediate airway neutrophil influx in both acute and chronic SO_2-induced bronchitis in the rat."

"These findings extend previous observations of PMNs as components of chronic bronchitis. PMNs might contribute to the pathogenesis of chronic bronchitis in a number of ways, including direct damage to respiratory epithelium or release of enzymatic or other mediators capable of altering the inflammatory milieu…the results from these studies suggest that the C-X-C chemokines KC and MIP-2 may contribute to the accumulation of airway PMNs during acute and chronic bronchitis."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Test animals:	**(+) but only female and sacrificed at end of experiment** Is the number of animals sufficient to produce statistically significant results? *Guideline: 5 animals/sex/dose level; if interim sacrifices are planned numbers should be increased.* Are animal characteristics adequately recorded and appropriate for the objective of the study? (+) a) Specie *Guideline: Rat, or justification for use of another mammalian specie.* (+) b) Age *Guideline: Adult, i.e.,8-12 weeks.* **(+) female only** c) Sex **(no info)** d) Pre-test health *Guideline: Females should be nulliparous and non-pregnant.* **(+) variation not given)** Weight variation *Guideline: Below 20% variation* ((+) f) Source *Guideline: An established breeding facility or educational institution*
B. Exposure conditions:	(-) Was a full description (manufacturer, purity, lot no.) of the reagents used in the trial provided?

	(-) Was there a gradient of exposure levels? *Guideline: Three exposure levels in addition to control.* (+) Were the chosen exposure levels appropriate to investigate the primary objective? (-) Were previous preliminary trials conducted to identify the range of appropriate exposure levels? **(not stated)** Were both nominal and actual exposure concentrations recorded? (+) Was the duration of exposure appropriate to investigate the primary objective? (+) Was the duration of exposure precisely defined? **(not stated)** If exposure chambers were employed, were animals exposed during the equilibration period? *Guideline: Animals should be placed in exposure chamber 4 hours after the chamber equilibrates.* (+) Was the mode of administration appropriate? (+) Was the frequency of administration appropriate to investigate the primary objective? (-) Was evidence provided to justify the dosing regime? **(not stated)** Were exposure concentrations, airflow, temperature, and humidity monitored continuously?
C. Housing/Feeding:	(+) Was the temperature, humidity, photoperiod, airflow and oxygen content maintained in the test and housing chambers appropriate? *Guideline: 22C (+/-3); 30-70%; 12h light/ 12h dark; 12 to 15 changes/h; 19%* (-) **not stated** Was the number of animals grouped in a single chamber stated? *Guideline: Groupings should permit clear observation of each animal.* **(not stated)** Was the manner by which animals were grouped appropriate? *Guideline: Animals may be grouped by sex, or individually.* (+) Was the type and source of feed and water stated? Was it appropriate? *Guideline: Conventional lab diet* **(+) but details not given for diet during exposure** Was the amount and feeding schedule specified? *Guideline: During housing, ad libitum with unlimited water; During exposure, food should be withheld and water may be withheld optionally.*

D. Equipment:	**(-)** Was information pertaining to the type, dimensions, material and atmospheric pressure of the exposure device outlined. *Guideline: Recommend oro-nasal or head exposure; slight negative pressure (< 5mm of water)* **(-)** Was a full description of the monitoring devices provided? **(not stated)** What was the sensitivity of the monitoring devices employed?
E. Procedural:	**(not specified)** Was the acclimation period specified? Was it of acceptable duration? *Guideline: 5 days* **(+)** Was a description of the pre-test conditions (*i.e.*, diet, quarantine, disease treatment, etc.) provided? **(not stated)** Were test animals randomly assigned to exposure groups? **(n/a)** Was the method of randomization defined? (*i.e.*, information pertaining to the mechanism used to generate random assignment) **(+)** Was a control group assayed? **(more detail needed)** Is the control regime appropriate? **(-)** Was the period of observation following exposure appropriate? *Guideline: Longer than 14 days.*
F. Data collection:	**(+)** Were appropriate endpoints examined? **(-(** Was the assessment thorough (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship was provided for each symptom)? **(+)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(n/a no symptom data)** Was the nature and severity of symptoms graded against an explicitly defined scale? **(not stated)** Did qualified personnel conduct the exams? **(+)** Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? **(-)** Was individual data reported for each test animal?
G. Data analysis:	**(+)** Are the statistical methods used appropriate? **(-)** Are confidence intervals reported? **(+)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?

H. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? **(more info needed)** Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable? **(more info needed)** Are the methods reproducible? **(more info needed)** Did either the study design or the statistical analysis limit confounding variables? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(no)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

In this study, the authors sought to investigate the mechanism underlying prominent airway neutrophil influx seen in a rat model of SO_2-induced chronic bronchitis. The findings indicate that PMNs are components of the inflammatory cells of chronic bronchitis and suggest that PMNs might contribute to the pathogenesis of chronic bronchitis in a number of ways, including direct damage to respirator epithelium in mice. Substantial increases were found in chemokine mRNA expression within tracheal tissue after acute and chronic exposures to SO2. These increases correlated with increases in PMN accumulation within tracheas, quantified in the acute exposures after immunohistochemical labeling of neutrophils. Similar PMN influx is seen morphologically in tracheas after longer SO_2 exposures.

Confidence index:

All of the rats were female and pretest health details are not provided.
There is limited information provided on the exposure conditions.
There is limited information on monitoring and exposure equipment.
There is no mention as to whether the animals are randomly exposures.
No mention was made of acclimation period.
Procedurally, this study did not fit the guidelines. Test animal information (age and pre-test health) was not provided and the number of subjects in this study were not sufficient to produce statistically significant results. This study does not explicitly rationalize the chosen exposure level and confidence levels are not given.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Holmen, A., Blomqvist, J., Frindberg, H., Johnelius, Y., Eriksson, N.E., Henricson, K.A., Herrstrom, P., Hogstedt, B.	**Internal ID:** 478

Title: Frequency of patients with acute asthma in relation to ozone, nitrogen dioxide, other pollutants of ambient air and meteorological observations.

Year: 1996

Abstract: OBJECTIVE: To study the association of the daily frequency of registration of patients with acute asthma at the emergency department of a central hospital in the south-west of Sweden with levels of air pollution and meteorological observations. METHODS: A retrospective longitudinal study was made of asthma patients taken from a hospital registry. This information was correlated with measurements of ozone, nitrogen dioxide, sulphur dioxide, toluene, temperature and relative humidity. Patients were from the catchment area of the Central Hospital of Halmstad containing around 120,000 inhabitants. A total of 4127 visits of patients with acute asthma to the emergency department at the Central Hospital of Halmstad were registered during a period of 1247 days from January 1990 to May 1993. The differential optical absorption spectroscopy (DOAS) technique was used to monitor levels of air pollutants over a distance of 1000 m in the central part of the town of Halmstad. Data on temperature, relative humidity, precipitation, wind speed and wind direction for the time period were supplied by the Swedish Meteorological and Hydrological Institute (SMHI). RESULTS: There were many statistically significant correlations between the levels of air pollutants and the meteorological measurements and a strong negative correlation between ozone and nitrogen dioxide. There was a statistically significant effect on asthma visits in children of low temperature and high nitrogen dioxide levels, and on asthma visits in adults of high temperature and high levels of ozone. CONCLUSIONS: There was a different reaction pattern in children and adults with asthma regarding temperature and ozone/nitrogen dioxide. The strong correlations between temperature and air pollution and between the levels of ozone and nitrogen dioxide made the true relation between asthma, air pollution and temperature hard to evaluate statistically.

Objective: To study the association of the daily frequency of registration of patients with acute asthma at the emergency department of a central hospital in the south-west of Sweden with levels of air pollution and meteorological observations.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
24 hr: Mean 4.9±5.7 μg/m^3 Range 0.2-78.4 μg/m^3 Day: Mean 5.2±6.6 μg/m^3 Range 0.2-81.6 μg/m^3 Night:	January 1990 – May 1993	Male and female	2990 visits – adults (age>15y mean 60.3) 1137 visits-children (age<15 years, mean 5.9)	Study population: ~120000 N=4127 visits by patients with acute asthma	5% of population suffers from asthma (as diagnosed by physician)	n/a	n/a

Mean 4.7±6.0 $\mu g/m^3$ Range 0.2-63.2 $\mu g/m^3$ Winter: Mean 6.7 $\mu g/m^3$ Summer: Mean 3.4 $\mu g/m^3$ Maximal 1h level reached during whole study period: 131 $\mu g/m^3$							

Observations:

During the summer there were significantly lower levels of SO_2.
The average number of asthma visits on working days was 2.3 and 2.7 on non-working days for adults ($p<0.0001$) and 1.1 and 0.6 respectively for children ($p<0.0001$).
The SO_2- values did not differ significantly between days with high and low frequency of asthma visits.
There were many statistically significant correlations between the levels of air pollutants (ozone, toluene, NO_2 and SO_2) and the meteorological measurements and a strong negative correlation between ozone and nitrogen dioxide.
No meteorological parameters showed any statistically significant differences between days with high and low asthma frequency in adults. There was no correlation between the number of asthma visits made by adults and children.

*Statistically significant

Author's Conclusions:

"There was a different reaction pattern in children and adults with asthma regarding temperature and ozone/nitrogen dioxide. The strong correlations between temperature and air pollution and between the levels of ozone and nitrogen dioxide made the true relation between asthma, air pollution and temperature hard to evaluate statistically."
**There were no specific conclusions about SO_2 effects given.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(Not enough information provided)** Is the number of subjects sufficient to produce statistically significant results? (+) **but not all data provided in paper** Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **Fixed monitors** Was the exposure concentration measured? (+) **Study period 1247 days** Is the exposure duration known?
C. Equipment:	(+) Was a full description of the monitoring devices provided? ($0.2\ \mu g/m^3$)What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(more info needed)** Were accepted assessment methods employed? (-) Were assessment measures sufficiently sensitive? **(more info needed)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (-) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+/-) **Appropriate, but more detail needed** Are the statistical methods used appropriate? (-) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective?

	(-) Were assumptions reasonable? (-) Are the methods reproducible? (some) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (none given) Do the conclusions make biological sense? (no extrapolations made) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no significant findings for SO_2 and daily frequency of hospital visits for asthma. This may be due to problems with the study design. See below.
The main finding in the study was a limited but statistically significant association in children between asthma visits and a winter-type climate/pollution situation with low temperature, high NO_2 and low ozone. Conversely, in adults asthma visits were associated with summer-type conditions with high temperature, high ozone and low NO_2. These associations were observed notwithstanding low levels of air pollution, especially NO_2.

Confidence index:

SO_2 measurements made 10m above ground and as such do not represent the conditions in the breathing zone of the patients.
Measurements of SO made in central part of Halmstad where pollution is at a higher level than the rest of the area. The monitoring process was very well described and the instruments were calibrated once every 3 months to ensure accuracy.
However, exposure assessment is a weakness of this study.
The diagnoses made by a nurse were not checked individually.
The introduction was very brief and the literature review was inadequate.
The description of the statistical methods was very brief.
The lack of a significant finding may be due to how the data were analyzed. The SO_2 levels were compared with high frequency and low frequency admission days. These days were arbitrarily defined by the authors as: for children (low: 0-1 visits/d high: 2-8 visits/day) and for adults (low: 0-3 visits/day and high: 4-12 visits/day). The difference between SO_2 levels on high frequency and low frequency days was determined not to be significant.
Most of the asthma cases in this region use medication for asthma which may have made them less susceptible.
There are approximately 8500 asthmatics calculated to be in the area; however, during the study period, only 1100 visits were recorded, less than 15%, implying that these cases have more severe asthma, and perhaps are more sensitive and less able at managing their disease with medication.
There is no discussion of SO_2 in the discussion section or the conclusion. There is minimal SO_2 information in the results section.
Some cases may visit their GP's office instead of emergency. These data were not included.
This study divided participants into age groups to account for the variability in immune system response between groups. While allergens may have played a role in respiratory symptomology for the spring months, this study was unable to account for allergen/pollen levels during this season.
Only one station was used to collect the pollution data for this experiment. Confidence intervals were not reported.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Kotesovec, F., Skorkovsky, J., Brynda, J., Peters, A., Heinrich, J. | **Internal ID:** 479

Title: Daily Mortality and Air Pollution in Northern Bohemia: Different Effects for men and women

Year: 2000

Abstract: The association between short-term changes in ambient air concentrations of TSP and SO_2 and day to day fluctuations in mortality was analyzed in the highly polluted Northern Bohemia region inhabited by approximately 630,000 people. A logistic regression model was adjusted for long term trends, seasonal cycles, influenza epidemics and weather parameters. The pollution and mortality data were available from 1982 to 1994. When the association was evaluated regardless of gender and age, 100 μg/m^3 increase of TSP, but not SO_2, was associated with a [illegible].4% increase of daily mortality lagged by 2 days. Evident differences in this association have been found between men and women. A significant increase in daily total and CVD mortality was observed in men below 65 while in women of the same age we found no association or even significant decrease in daily mortality. For the population over 65 the differences between genders were again apparent. The mortality in women increased significantly while in men significant decrease was demonstrated. CONCLUSIONS: The evaluation with respect to gender might contribute to identifying susceptible subgroups.

Objective: This paper presents some results concerning the association between daily SO_2 and TSP concentrations and day to day fluctuations in mortality for men and women in Northern Bohemia.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Mean SO_2 : Whole study period: 99.8 ± 89.5 μg/m^3 Range: 8.5-986.7 μg/m^3	Study period: 1982-1994	Male and female	All ages	Total counts of deaths (all causes) : Male: 45074 Female: 41206 Cardiovascular deaths: Male:24064 Female: 25320 Respiratory deaths: Male: 12684 Female: 9199	No info	n/a	n/a

Observations:

When the association between daily total mortality and air pollution for the entire population was evaluated regardless of gender, age, and cause of death, SO_2 concentrations were not significantly associated with mortality. The risk ratios were calculated for a 100 $\mu g/m^3$ increase in SO_2.

When the association between daily cancer mortality and ambient air pollution was evaluated with respect to gender, only the cancer mortality of males was significantly higher in association with increasing SO_2 concentrators (lags 0, 1, and 4).

No association between SO_2 and respiratory mortality was found for the whole population, for men or for women.

In the over 65 years age group, when the association between daily cancer mortality and ambient air pollution was evaluated with respect to gender, only the cancer mortality of males was significantly higher in association with increasing TSP and SO_2 concentrations.
In the elderly (>65y), the daily total and CVD mortality evaluated regardless of gender did not exhibit any significant increase with SO_2 concentrations. Only the cancer mortality of women increased significantly for lag 5 with SO_2, RR=1.04 (1.011-1.083) for a 100$\mu g/m^3$ increase in SO_2.

In the younger population ($\leq$65 y) the daily total mortality, irrespective of gender, significantly increased in association with SO_2 lag 4 and 6. Whereas the mortality of men increased significantly with SO_2 (lag 1, 3, 4, 6, 7 day mean), the RR for women was always below one, for SO_2.
The significant increase in the daily mortality (CVD) of males was found in association with SO_2 (lag 3, 4, 6, 7 day means). The daily RR in females was again below one with a significant decrease in association with SO_2 lag 2. For cancer mortality, there was a significant increase in daily mortality in association with SO_2 for lag 4. The RR for cancer caused deaths in women was below one and a significant decrease was seen with SO_2 (lag 5, 6, 7 day means).

Author's Conclusions:

"A significant positive association between air pollution and all cause mortality was found when evaluated regardless of gender and age. A more detailed evaluation, when gender and age were considered, has shown that the increased mortality of males below 65 and, to a lesser extent, women over 65, were predominantly responsible for this association."
"The results of our study indicate that there are considerable gender differences in the association between daily mortality and short-term changes in ambient air pollution. The evaluation with respect to gender, which should be replicated in other data sets, might bring new information and contribute to identifying the most vulnerable subgroups."
"Only cancer mortality was positively significantly associated with air pollution. This finding is rather surprising, because this group of diagnoses comprises all types of cancer and only a few of them, especially cancer of the respiratory system are believed to be associated with short term exposure to air pollution. It is of course possible that the immediate cause of death, both in patients with respiratory carcinomas and with CVD, was pneumonia that was not mentioned in death certificates. The possible underreporting of pneumonia in death certificates might be partially responsible for the low incidence of respiratory disease mortality, so that the role of short-term exposures to air pollution could not be observed."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	. (+)Is the number of subjects sufficient to produce statistically significant results?

	(-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? **(moreinfo needed)** Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **fixed monitors**Was the exposure concentration measured? (+) **study period 1982-1994** Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? (+) **mortality counts** Were appropriate endpoints examined? (+) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) **mortality counts/ICD 9 codes**Were accepted assessment methods employed? (+) Were assessment measures sufficiently sensitive? **(n/a mortality counts)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? **(some)** Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (-) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)?

	. (more info needed) Do the conclusions make biological sense? . (+) Are the extrapolations reasonable? . (+) Is the paper in a peer-reviewed journal? . (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

The association between short term changes in ambient air concentrations of SO_2 and day to day fluctuations in mortality was analyzed. When the association was evaluated regardless of gender and age, there was no significant association between $SO_2$and mortality... A significant increase in daily total and CVD mortality was observed in men below 65 while in women of the same age there was no association or even a significant decrease in daily mortality. For the population over 65 the differences between genders were again apparent. The mortality in women increased significantly while in men significant decrease was demonstrated. Further evaluation with respect to gender might bring new information and contribute to identifying the most vulnerable subgroups.

Confidence index:

The geography of the study area is very heterogeneous and could have affected the exposure. This study utilized a community that experiences high concentrations of air-borne pollutants based on the coal industries located in the region. However, only two monitoring stations were available for each region, not representative of the large area involved. Exposure assessment is a weakness of this study.

There were missing SO_2 data from the stations throughout the study period. There are no specific details on how much data were missing and how much data were therefore estimated.

The significant relative risks reported for the effects of SO_2exposure on cancer mortality in men were actually close to null and RR=1 was included in some of the confidence intervals. This is also the case for the adjusted relationship between daily total mortality and CVD mortality and daily means of air pollutants for the younger age group. Most of the 95% confidence intervals include RR=1.00 or were very close to one. Too many significant figures are reported for the relative risks, eg. 95% CI = 1.002-1.067 RR=1.034 reported as significant. The adjusted relationship between daily cancer mortality and daily means of air pollution included significant associations as reported by the authors. However, again, the RR's were close to null and the 95% intervals contained RR=1 for men. The results for women are inconsistent. SO_2 is reported as having a significant negative association with daily cancer mortality in woman <65y, RR=0.918-0.942.

The control for confounders was explained in detail.

One possible limitation of the study design is that there may have been underreporting of pneumonia on death certificates, so that the role of short term exposures to air pollution could not be observed.

Mortality data was acquired from several different sources, from databases to autopsy. This may have introduced bias into the study. Possible confounding factors such as influenza epidemics, short-term calendar effects and meteorology were accounted for.

Gender differences such as lifestyle and genetics could contribute to modification of the association between air pollution and mortality.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Hong, Y.C., Leem, J.H., Ha, E.H.	**Internal ID:** 480
Title: Air Pollution and Daily Mortality in Inchon, Korea	
Year: 1999	
Abstract: The association between total daily mortality and air pollution was investigated for a 1-year period (January 1995 to December 1995) in Inchon, Korea. The purpose of this study was to evaluate the relative importance of particulate and gaseous air pollution as predictors of daily mortality. Concentration of total suspended particulates (TSP), inhalable particles (PM_{10}), and gaseous pollutants, such as sulfur dioxide, nitrogen dioxide, ozone, carbon monoxide, were measured daily during the study period. A generalized additive model was used to regress daily death counts on each air pollutant, controlling for time trend and meteorologic influences such as temperature or relative humidity. Total mortality was found to increase 1.2% (95% CI: 0.2 to 2.2%) for each 10 $\mu g/m^3$ increase in 6-day moving average of TSP, and 1.2% (95% CI 0.2 to 2.1%) for each 10 $\mu g/m^3$ increase in 5-day moving average of PM_{10}. The association is similar in magnitude to associations between particulate air pollution and mortality found in several other communities in America and Europe. Associations with gaseous pollutants were all statistically insignificant in the generalized additive model. The relative risk of death increased at particulate levels that were well below the current Korean Ambient Air Quality Standard.	
Objective: The objective of the present study was to report on the association between daily mortality and daily levels of ambient air pollution in Inchon.	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
22.6 ppb (mean) 7.2-52.8 ppb (range)	Jan 1995 - Dec 1995 Daily exposure over a one-year period	Male and female	varied	Inchon (2.2 million)	varied	unknown	unknown

Observations:

The correlations between the daily mortality and pollution variables were small but significant.
The relative risk for an increase of 10 $\mu g/m^3$ SO_2 and daily mortality, controlling for time trend and weather variables was not significant (RR=1.007 (0.56-1.062).
The relative risk for an increase of 10 $\mu g/m^3$ SO_2 and daily cardiovascular mortality, controlling for time trend and weather variables was not significant (RR=1.028 (0.937-1.129)

Author's Conclusions:

There were no specific conclusions regarding SO_2 effects.
"NO_2, SO_2, and CO are gaseous pollutants released mainly from motor vehicle and combustion sources. Our analysis showed that gaseous pollutants were not significantly associated with daily mortality and the association of gases remained insignificant in two pollutants model controlling particulate pollutant. This

result was similar with previous reports which described insignificant association between mortality and gases, when particulate pollutants were included in the model. It is well known that NO_2 and SO_2 are converted to sulfates and nitrates thus contributing to the fine particles. That makes the individual effect of the gaseous pollutants more difficult to explain."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+)Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (More info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Fixed monitors Was the exposure concentration measured? (+) Study period 365 days Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (Not provided) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc.) (+) Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc.)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (+)Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+)Were accepted assessment methods employed? (+/-) Were assessment measures sufficiently sensitive? (n/a mortality counts) Was the nature and severity of symptoms graded against an explicitly defined scale? (No info provided) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (some) Was raw data provided to permit the reader to arrive at his/her own conclusions? (n/a mortality counts) Was individual data reported for each subject? (n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+)Are the statistical methods used appropriate? (+)Are confidence intervals reported?

	(+)Did the investigator appropriately interpret the statistics? (-)Are the observed trends statistically significant?
G. Interpretations:	(+)Was the original objective addressed? (-)Are the results novel? **(more info needed)** Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable? (-)Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(n/a no conclusion made)** Do the conclusions make biological sense? **(n/a no extrapolations made)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no significant findings regarding the association between SO_2 and mortality. These results may be due to problems with the study design.

Findings indicated that gaseous pollutants such as SO_2 were not significantly associated with daily mortality and the association of gases remained insignificant in a two-pollutant model controlling particulate pollutant.

Confidence index:

The ICD codes used for inclusion of cases of mortality into the study were not given.

There is insufficient detail on the population from which the mortality data was collected.

There is no detail given on the methods of handling missing pollution data.

More detail of the statistical analysis is needed.

Though there is no significant relationship detected for SO_2, there is no real discussion on the probable reasons for this. The authors report consistency with other studies, but give no references supporting these findings.

The validity of the findings from this study is called into question by the limited number of monitoring stations used to record data in the third largest city in Korea. Furthermore, the Department of Environment determined where the monitoring stations were to be set up, instead of allowing study authors to exercise control over the data collection process.

Only 3 monitoring stations were used to measure SO_2 over 1 year for a population of 2.2 million.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: Wong, T.W., Lau, T.S., Yu, T.S., Neller, A., Wong, S.L., Tam, W., Pang, S.W.	**Internal ID:** 481

Title: Air Pollution and hospital admissions for respiratory and cardiovascular diseases in Hong Kong

Year: 1999

Abstract: OBJECTIVE: To investigate short term effects of concentrations of pollutants in ambient air on hospital admissions for cardiovascular and respiratory diseases in Hong Kong. METHODS: Retrospective ecological study. A Poisson regression was performed of concentrations of daily air pollutant on daily counts of emergency hospital admissions in 12 major hospitals. The effects of time trend, season, and other cyclical factors, temperature, and humidity were accounted for. Autocorrelation and overdispersion were corrected. Daily concentrations of nitrogen dioxide (NO_2), sulphur dioxide (SO_2), ozone (O_3), and particulate matter < 10 microns in aerodynamic diameter (PM_{10}) were obtained from seven air monitoring stations in Hong Kong in 1994 and 1995. Relative risks (RR) of respiratory and cardiovascular disease admissions (for an increase of 10 $\mu g/m^3$ in concentration of air pollutant) were calculated. RESULTS: Significant associations were found between hospital admissions for all respiratory diseases, all cardiovascular diseases, chronic obstructive pulmonary diseases, and heart failure and the concentrations of all four pollutants. Admissions for asthma, pneumonia, and influenza were significantly associated with NO_2, O_3, and PM_{10}. Relative risk (RR) for admissions for respiratory disease for the four pollutants ranged from 1.013 (for SO_2) to 1.022 (for O_3), and for admissions for cardiovascular disease, from 1.006 (for PM_{10}) to 1.016 (for SO_2). Those aged > or = 65 years were at higher risk. Significant positive interactions were detected between NO_2, O_3, and PM_{10}, and between O_3 and winter months. CONCLUSIONS: Adverse health effects are evident at current ambient concentrations of air pollutants. Further reduction in air pollution is necessary to protect the health of the community, especially that of the high risk group.

Objective: To investigate short term effects of concentrations of pollutants in ambient air on hospital admissions for cardiovascular and respiratory diseases in Hong Kong.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Daily Median: 17.05 $\mu g/m^3$ Range: 2.74-68.49 $\mu g/m^3$	Study period: 1994-1995	Male and female	No info	Respiratory Admissions: 84-232/day Cardiovascular Admissions: 54-177/day	No info	N/a	n/a

Observations:

Significant associations were found between admissions for respiratory diseases and an increase of 10 $\mu g/m^3$ for SO_2. For the age group $\geq$65yr, current day exposure, the RR=1.023, 95% CI 1.012-1.036). For all ages, the RR=1.013 95% CI 1.004-1.021).

Significant associations were found between admissions for cardiovascular diseases and an increase of 10 $\mu g/m^3$ for SO_2. For the $\geq$65yr group, current day and previous day exposure, the RR=1.021 95% 1.010-1.032). For all ages, the RR=1.016 95% CI 1.006-1.026.

For individual diseases, significant associations were found between admissions for Chronic obstructive pulmonary diseases and an increase of 10 $\mu g/m^3$ increase in SO_2 : For current day exposure, RR=1.023 95% CI = 1.011-1.035. For heart failure, the results were also significant for current day exposure, RR=1.036 95% CI 1.013-1.059.

Author's Conclusions:

"Adverse health effects are evident at current ambient concentrations of air pollutants. Further reduction in air pollution is necessary to protect the health of the community, especially that of the high risk group. Interactions among pollutants and their pathophysiological mechanisms remain to be clarified. Despite our incomplete understanding, stricter control of air pollutants at source should lower the concentrations of the ambient pollutants and thus reduce mortalities and morbidities."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(+) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitorsWas the exposure concentration measured? (+) Study period 1994-1995 Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? (no info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc.) (+) Was the timing and length of follow-up appropriate? (+) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc.)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (more info needed) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and

	dose-response relationship provided for each symptom) **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(no info)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (+)Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? **(n/a no withdrawals reported)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? (+) Were assumptions reasonable? (-) Are the methods reproducible? **(some)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **No specific SO_2 conclusions** Do the conclusions make biological sense? **no extrapolations made** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (-) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Significant associations were found between hospital admissions for all respiratory diseases, all cardiovascular diseases, chronic obstructive pulmonary diseases and heart failure and SO_2. When the data was stratified by ages, only the >65yrs age group had significant associations. Those aged ≥65y accounted for 68% and 38% of admissions for cardiovascular and respiratory diseases respectively. Children under 5 accounted for 31% of respiratory diseases but only 0.5% of cardiovascular diseases. Therefore, statistical power may not have been sufficient in some of the age groups. However, the identification of target diseases and high risk groups would be useful in finding suitable air quality guidelines in env. health.

The author notes that, as with all ecological studies, the study is limited by the lack of precise exposure estimates. Furthermore, caution should be exercised in inferring cause-effect relations. Findings from this study suggest that current concentrations of air pollutants may produce health effects.

Adverse respiratory effects of SO_2 were detectable even at relatively low concentrations. Significant associations with cardiovascular morbidities have not been as widely reported elsewhere as respiratory illnesses. Authors suggest that caution should be exercised in inferring cause-effect relations.

Confidence index:

The data contained too many significant figures. The authors report that compared with respiratory and cardio admissions, the RR for certain disease coding such as asthma, COPD and heart failure were higher and seemed to be more sensitive indicators of health effect, whereas other diseases showed no association. However, the reported differences in the RR were in some causes as small as 0.002.

There was no information on the location of the monitors with respect to the population. There was very limited information on the measurement of SO_2 data. As with all ecological studies, this study is limited by the lack of precise exposure estimates.

Most confounders were established and the authors used the well-established statistical methods of the APHEA protocol.

This study used a large sample size. Description information such as socioeconomic data or previous health history were not collected. Confidence intervals were reported..

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Sheppard, L., Levy, D., Norris, G., Larson, T.V., Koenig, J.Q.	**Internal ID:** 482

Title: Effects of Ambient Air Pollution on Nonelderly Asthma Hospital Admissions in Seattle, Washington, 1987-1994

Year: 1999

Abstract: As part of the Clean Air Act, Congress has directed EPA to set air quality standards to protect sensitive population groups from air pollutants in the ambient environment. People with asthma represent one such group. We undertook a study of the relation between measured ambient air pollutants in Seattle and nonelderly hospital admissions with a principal diagnosis of asthma. We regressed daily hospital admissions to local hospitals for area residents from 1987 through 1994 on particulate matter less than 10 and 2.5 μm in aerodynamic diameter (PM_{10} and $PM_{2.5}$, respectively); coarse particulate mass; sulfur dioxide (SO_2); ozone (O_3); and carbon monoxide (CO) in a Poisson regression model with control for time trends, seasonal variations, and temperature-related weather effects. With the exception of seasonally monitored O_3, we supplemented incomplete pollutant measures in a multiple imputation model to create a complete time series of exposure measures. We found an estimated 4-5% increase in the rate of asthma hospital admissions associated with an interquartile range change in PM (19 $\mu g/m^3$ PM_{10}),11.8 microg/m3 $PM_{2.5}$, and 9.3 microg/m3 coarse particulate mass) lagged 1 day; relative rates were as follows: for PM_{10}, 1.05 [95% confidence interval (CI) = 1.02-1.08]; for $PM_{2.5}$, 1.04 (95% CI = 1.02-1.07); and for coarse particulate mass, 1.04 (95% CI = 1.01-1.07). In single-pollutant models we also found that a 6% increase in the rate of admission was associated with an interquartile range change in CO (interquartile range, 924 parts per billion; 95% CI = 1.03-1.09) at a lag of 3 days and an interquartile range change in O_3 (interquartile range, 20 parts per billion; 95% CI = 1.02-1.11) at a lag of 2 days. We did not observe an association for SO_2. We found PM and CO to be jointly associated with asthma admissions. We estimated the highest increase in risk in the spring and fall seasons.

Objective: We undertook a study of the relation between air pollution in Seattle and nonelderly hospital admissions for asthma from 1987 through 1994. We evaluated the hypothesis that the association between asthma admissions and air pollutants will be stronger during relatively high wood smoke exposure periods.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Mean: 8.0 ppb	Study period: 1987-1994	Male and female	<65 yrs	Average hospital admissions for asthma: 2.7/day During the study period: 7837 new asthma admissions 6437 appendicitis admissions (serving as control)	No info	n/a	n/a

Observations:

54% of all admissions were 19 years old or younger.
Associations with SO_2 were not observed. The increase in SO_2 that was used for this analysis was approximately 10 ppb. However, appendicitis admissions,

which were supposed to serve as a control, showed an association for SO_2 levels. The rest of the observations looked at combinations of the pollutants. Exposure to SO_2 was low and was due almost exclusively to emissions from local sources in the vicinity of the monitor. SO_2 and O_3 showed only weak correlations with all other pollutants. For asthma admissions, a 4-6% increase in admissions per interquartile range increment of all pollutants except SO_2.

Author's Conclusions:

There were no specific conclusions regarding SO_2 effects.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (more info needed) Are the subjects a representative sample of the population to which extrapolations will be made? (+) Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) Fixed monitorsWas the exposure concentration measured? (+) Study period 8 years Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (No info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (+) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (more info needed) Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	(n/a) Was the assessment identical for cases and controls? (more info needed) Were appropriate endpoints examined? (-) Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? (-) Were assessment measures sufficiently sensitive? (more info needed) Was the nature and severity of symptoms graded against an explicitly defined scale? (+) Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject?

	(n/a no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (+) Was the design of the study appropriate to investigate the primary objective? **(more info needed)** Were assumptions reasonable? (-) Are the methods reproducible? (+) Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(n/a no conclusions re SO_2)** Do the conclusions make biological sense? **(n/a no extrapolations)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (no) Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

There were no associations found for SO_2 levels and asthma admissions. Surprisingly there was an association between a 10 ppb increase in SO_2 and appendicitis admissions, which were the control outcome.

Confidence index:

The focus of this study was on particulate and black smoke. There was very little detail on SO_2 analysis.
The literature review/introduction was extensive.
Statistical significance was not reported for the data.
SO_2 concentration data was only collected from 3 sites. This study provides information on the number and location of all monitors used for the study.
Approximately 6% of the data for SO_2 concentrations was missing. The authors explained in great detail the methods for estimating the values for the missing data. However it seemed like there was extensive data manipulation. Exposure assessment is a weakness of this study design.
All methods of analysis were justified by referencing other studies.
Possible reasons or discussion regarding the lack of an association for SO_2 and asthma admissions were not provided.
A map was provided showing the locations of the monitors.
This study used as a control group all nonelderly admissions for appendicitis. The exclusion criteria for this study was clearly outlined in the study - all elective, newborn, transfer and law enforcement admissions were excluded.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Schwartz, J., Dockery, D.W.	**Internal ID:** 483

Title: Increased Mortality in Philadelphia Associated with Daily Air Pollution Concentrations

Year: 1992

Abstract: Cause-specific deaths by day for the years 1973 to 1980 in Philadelphia, Pennsylvania, were extracted from National Center for Health Statistics mortality tapes. Death from accidents (International Classification of Disease, Revision 9 greater than or equal to 800) and deaths outside of the city were excluded. Daily counts of deaths were regressed using Poisson regression on total suspended particulate (TSP) and/or SO_2 on the same day and on the preceding day, controlling for year, season, temperature, and humidity. A significant positive association was found between total mortality (mean of 48 deaths/day) and both TSP (second highest daily mean, 222 micrograms/m^3) and SO_2 (second highest daily mean, 299 micrograms/m^3). The strongest associations were found with the mean pollution of the current and the preceding days. Total mortality was estimated to increase by 7% (95% CI, 4 to 10%) with each 100-micrograms/m^3 increase in TSP, and 5% (95% CI, 3 to 7%) with each 100-micrograms/m^3 increase in SO_2. When both pollutants were considered simultaneously, the SO_2 association was no longer significant. Mortality increased monotonically with TSP. The effect of 100 micrograms/m^3 TSP was stronger in subjects older than 65 yr of age (10% increase) compared with those younger than 65 yr of age (3% increase). Cause-specific mortality was also associated with a 100-micrograms/m^3 increase in TSP: chronic obstructive pulmonary disease (ICD9 490-496), +19% (95% CI, 0 to 42%), pneumonia (ICD9 480-486 & 507), +11% (95% CI, -3 to +27%), and cardiovascular disease (ICD9 390-448), +10% (95% CI, 6 to 14%). These results are somewhat higher than previously reported associations, and they add to the body of evidence showing that particulate pollution is associated with increased daily mortality at current levels in the United States.

Objective: This report presents an analysis of daily total and cause-specific mortality in Philadelphia, Pennsylvania, compared with daily TSP and SO_2 concentrations for 8 years.

Overall study design:

Exposure level	**Exposure frequency/duration**	**Gender**	**Age**	**Number of subjects**	**Pre-trial health**	**NOAEL**	**LOAEL**
Mean 21 ppb	1973-1980	Male and female	65% of deaths over total study period: >65yr	Population at 1980: 1688710 Average deaths/day: Cardiovasucalu:22.1 Cancer: 10.5 Pneumonia:1.44 COPD: 0.89	No info	n/a	n/a

Observations:

A significant positive association was found between total mortality and both TSP and SO2. Current day and prior day SO_2 measurements (ppb) were significant predictors of daily mortality. (No further data as far as RR or pollutant values are provided)

A significant positive association was found between total mortality (mean of 48 deaths/day) and SO_2 (second highest daily mean, 299 µg/m^3). The strongest associations were found with the mean pollution of the current and the preceding days. Total mortality was estimated to increase by 5% (95% CI, 3-7%) with each 100 μg/m^3 increase in SO_2. When TSP and SO_2 were considered simultaneously, the SO_2 association was no longer significant.

Author's Conclusions:

There were no conclusions regarding SO_2 specifically.

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(more info needed) Is the number of subjects sufficient to produce statistically significant results? (-) Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? (n/a no controls) Were identical 'criteria for exclusion' applied to both cases and controls? (+)Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) fixed monitors Was the exposure concentration measured? Study period 8 years Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? (No info) What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? (n/a no controls) Were appropriate controls recruited? (n/a no blinding) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc.) (+) Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc.)
E. Data collection:	(n/a no controls) Was the assessment identical for cases and controls? (more info needed) Were appropriate endpoints examined? (-)Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (more info needed) Were accepted assessment methods employed? (more info needed)Were assessment measures sufficiently sensitive? (+) Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+)Was the timing and frequency of assessment logical? (-)Was raw data provided to permit the reader to arrive at his/her own conclusions? (n/a mortality counts) Was individual data reported for each subject? (no withdrawals) Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(more info needed) Are the statistical methods used appropriate?

	(-) Are confidence intervals reported? (Not enough detail provided) Did the investigator appropriately interpret the statistics? (Not enough detail provided) Are the observed trends statistically significant?
G. Interpretations:	(+/-)Was the original objective addressed? (-)Are the results novel? (-)Was the design of the study appropriate to investigate the primary objective? (more info needed) Were assumptions reasonable? (-) more info needed Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (n/a no conclusions re SO_2) Do the conclusions make biological sense? (no extrapolations made) Are the extrapolations reasonable? (+)Is the paper in a peer-reviewed journal? (No)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

A significant positive association was found between total mortality (mean of 48 deaths/day) and SO_2 (second highest daily mean, 299 micrograms/m^3). The strongest associations were found with the mean pollution of the current and the preceding days. Total mortality was estimated to increase by 5% (95% CI, 3 to 7%) with each 100-micrograms/m^3 increase in SO_2.

The study's authors recommend that further studies would be useful to confirm the apparently causal relationship between air particles and daily mortality.

Confidence index:

The findings noted above are from the abstract. There are no details regarding the SO_2 associations with mortality in the body of the paper. This makes it difficult to give an accurate assessment of the study.

15 samplers were used to collect data from around the city of Philadelphia. However, SO_2 levels are reported as the mean for the 8 year period.

About 99% of the results section discusses total suspended particulate. Exposure assessment is a weakness of this study.

This is an ecological study and the authors report their findings regarding TSP to be likely causal.

This study notes that accidental deaths and all deaths occurring outside of the city were excluded from the mortality data used in the study. While cause of death was recorded, other information such as socioeconomic status and previous health information was not.

Compared to other ecological studies, this report is good. Conclusions are well supported and make biological sense and extrapolations are reasonable for particulates. However, ecological study designs do not have the power to make conclusions of causal associations. Ecological study designs can generate hypotheses, not test them.

Review – Confidence index ranking:

Low to moderate

CASE-CONTROL REVIEW FORM

Author: Castellsague, J., Sunyer, J., Saez, M., Anto, J.M.	**Internal ID:** 484
Title: Short-term association between air pollution and emergency room visits for asthma in Barcelona	
Year: 1995	
Abstract: BACKGROUND--Several studies have assessed the association between urban air pollutants and hospital admissions or emergency room visits for asthma with inconsistent results. The objective of this study was to assess the relation between levels of black smoke, sulphur dioxide, nitrogen dioxide, and ozone and adult emergency room visits for asthma in Barcelona, Spain during the five-year period 1985-9. METHODS--The daily number of emergency room visits for asthma was obtained from a register of respiratory emergencies designed to study the asthma outbreaks occurring in Barcelona. The association between asthma visits and levels of pollutants was assessed separately for summers and winters with Poisson regression models controlling for meteorological and time related variables. RESULTS--Black smoke was associated with asthma visits in summer but not in winter. The relative risk (RR) of asthma visits for a 25 $\mu g/m^3$ increase of current day concentrations of black smoke was 1.082 (95% CI 1.011 to 1.157). The mean current and previous three day levels of black smoke led to a stronger association (RR = 1.114 (95% CI 1.010 to 1.160). In addition, nitrogen dioxide was associated with asthma visits in both summer (RR = 1.045, 95% CI 1.009 to 1.081) and winter (RR = 1.056, 95% CI 1.011 to 1.104). These associations were slightly higher for the previous day's level of nitrogen dioxide. No associations were found for sulphur dioxide or for ozone. CONCLUSIONS--This study provides further evidence of the effect of particulate pollution on asthma, and it suggests that nitrogen dioxide may have a role in the exacerbation of bronchial asthma in adults.	
Objective: The objective of this study was to assess the relation between levels of black smoke, sulphur dioxide, nitrogen dioxide, and ozone and adult emergency room visits for asthma in Barcelona, Spain during the five year period 1985-9	

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Means Summer: 40.8ppb Winter: 52.0 ppb	Study period: 1985-1989 (460 days)	Male and female	>14years		Asthmatic	n/a	n/a

Observations:

Sulphur dioxide for both seasons showed positive although not statistically significant associations with asthma visits. Relative risks were calculated for a $25 \mu g/m^3$ increase.

Author's Conclusions:

There were no conclusions regarding SO_2 levels and emergency room visits from this study.

Stated conclusions are unclear:

> "In our study neither sulphur dioxide are consistent with the results of Goldstein et al. in New York where no association was observed at levels considerably higher than those of Barcelona. Similarly, in the Seattle study sulphur dioxide was not related with emergency room visits for asthma, although in this city, the station monitoring sulphur dioxide levels was not representative of the population exposure. In contrast, some studies have reported significant associations between sulphur dioxide levels and admissions or emergencies for asthma. Levels of sulphur dioxide have been related to hospital admissions for asthma in Ontario in the summer but not in winter, and in both seasons in Vancouver. Significant associations have also been reported in winter in Birmingham, a city with lower sulphur dioxide levels than Barcelona. In addition, most controlled chamber studies have consistently shown that asthmatics are more sensitive to sulphur dioxide than healthy subjects."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	(-) Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? **(more info needed)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **fixed monitors**Was the exposure concentration measured? (+) **study period 460 days** Is the exposure duration known?
C. Equipment:	(-) Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-) Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? (-) Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+) Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? **(more info needed)** Were appropriate endpoints examined? **(more info needed)** Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) (+) Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? (-) Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical?

	(some) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-)Was individual data reported for each subject? **(n/a no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(+) Are the statistical methods used appropriate? (+) Are confidence intervals reported? (+) Did the investigator appropriately interpret the statistics? (-) Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-)Are the results novel? (+)Was the design of the study appropriate to investigate the primary objective? (+)Were assumptions reasonable? (-) Are the methods reproducible? (+)Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? **(No conclusions re SO_2)** Do the conclusions make biological sense? **(no extrapolations re SO_2 made)** Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? (No)Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

No associations were found for sulphur dioxide.

Confidence index:

An admission for asthma was defined as an emergency room visit during which any asthma related diagnosis. A panel of respiratory physicians developed a list of equivalent terms used by clinicians in the diagnosis of asthma. An alternative list for COPD was also used and all visits recorded under this category were excluded. This method of choosing cases could have led to misclassification of outcome.

The monitoring system was extensive, 15 manual samplers and three automatic stations. However, there was no detail given on the monitoring and measurement of SO_2. Exposure assessment is a limitation of this study.

More detail is needed in the statistical analysis section.

Some confounders such as weather, concentration of ambient allergens, respiratory viral infections, or patients' activity patterns, autocorrelation and over dispersion were considered in the model.

No data were available concerning socioeconomic information or prior health history.

Review – Confidence index ranking:

Low

CASE-CONTROL REVIEW FORM

Author: **Garty, B.Z., Kosman, E., Ganor, E., Berger, V., Garty, L., Wietzen, T., Waisman, Y., Momouni, M., Waisel, Y.**

Internal ID: 485

Title: Emergency room visits of asthmatic children, relation to air pollution, weather, and airborne allergens

Year: 1998

Abstract: BACKGROUND: The worldwide increase in the incidence, prevalence, and severity of asthma may suggest that environmental factors play a role in these epidemiologic changes. OBJECTIVE: To examine the correlations between air pollutants, weather conditions, airborne allergens, and the incidence of emergency room (ER) visits of children with acute asthma attacks. DESIGN: One-year prospective study. Data of daily concentration of air pollutants, weather conditions, and selective airborne allergens were collected and compared with the number of ER visits of asthmatic children. SUBJECTS: 1076 asthmatic children (aged 1 to 18 years) who presented at the Pediatric ER between January 1 and December 31, 1993. RESULTS: Correlations between fluctuations in ER visits of asthmatic children and various environmental parameters were more relevant for weekly than for daily values. Emergency room visits correlated positively with concentrations of NOx, SO_2 and with high barometric pressure; and negatively with O_3 concentration and minimal and maximal temperature. There were no significant correlations with concentrations of particulates, humidity, or airborne pollen and spores. An exceptionally high incidence of ER visits of asthmatic children was observed during September. This peak coincided with the beginning of the school year and the Jewish holidays. The correlations between ER visits and the environmental factors increased significantly when the September peak was excluded, revealing that 61% of the variance in ER visits was explained by NOx, SO_2, and O_3 concentrations, 46% by weather parameters, 66% by NOx, SO_2 and barometric pressure, and 69% by the combination of air pollutants and weather parameters. CONCLUSION: The major factors found to be associated with ER visits of asthmatic children were high NOx, high SO_2, and high barometric pressure. Negative correlation was found between ER visits of asthmatic children and ozone concentrations. The particularly high number of ER visits at the beginning of the school year and the Jewish holidays was probably associated with an increase in the number of viral infections and/or emotional stress.

Objective: To examine the correlations between air pollutants, weather conditions, airborne allergens, and the incidence of emergency room (ER) visits of children with acute asthma attacks.

Overall study design:

Exposure level	Exposure frequency/duration	Gender	Age	Number of subjects	Pre-trial health	NOAEL	LOAEL
Weekly mean SO_2: approx. 10-65 μg/m^3	Study period: January 1 – December 31, 1993	Male-664 Female-412	1-18 yr	1076	No info	n/a	n/a

Observations:

A positive correlation was found between ER visits and the mean ambient concentrations of SO_2.

The deviation from the mean ER visits during the entire year is expressed by variance. Approximately 28% of the variance in the number of ER visits was explained by fluctuations in SO_2.

All other observations looked at the effects of all pollutants together, not SO_2 independently. Correlations between fluctuations in ER visits of asthmatic children and various environmental parameters were more relevant for weekly than for daily values.

Statistical significance was not calculated.

Author's Conclusions:

"The major factors found to be associated with ER visits of asthmatic children were high NOx, high SO_2, and high barometric pressure. The particularly high number of ER visits at the beginning of the school year and the Jewish holidays was probably associated with an increase in the number of viral infections and/or emotional stress."

Review – Assessment: [NB: In actual reviews ! is replaced by: (+) denoting compliance with guidelines, (-) denoting a diversion from guidelines or (+/-) denoting a characteristic which is neither positive nor negative but is worthy of mention.]

A. Subjects:	**(more info needed)** Is the number of subjects sufficient to produce statistically significant results? (-)Are characteristics (*e.g.*, age, sex, health, work history, medical history) of test subjects adequately recorded and appropriate for the objective of the study? (-)Are the subjects a representative sample of the population to which extrapolations will be made? **(n/a no controls)** Were identical 'criteria for exclusion' applied to both cases and controls? **(more info needed)** Was the method of recruitment appropriate? Would the participation incentive employed cause the selection of an unrepresentative sample population?
B. Exposure conditions:	(+) **Fixed monitors**Was the exposure concentration measured? (+) **Study period 1 year**Is the exposure duration known?
C. Equipment:	(-)Was a full description of the monitoring devices provided? **(no info)** What was the sensitivity of the monitoring devices employed?
D. Procedural:	(-)Was a precise definition of what constitutes 'exposure' outlined before commencement of the data retrieval? **(n/a no controls)** Were appropriate controls recruited? **(n/a no blinding)** Was the type of blinding indicated? (*i.e.*, single, double, triple, etc) (+)Was the timing and length of follow-up appropriate? (+)Was the nature of follow-up appropriate? (*i.e.*, questionnaire, interview, medical exam, etc)
E. Data collection:	**(n/a no controls)** Was the assessment identical for cases and controls? **(more info needed)** Were appropriate endpoints examined? **(more info needed)** Was the assessment thorough? (*i.e.*, information pertaining to the clinical nature, duration, reversibility, severity, onset and dose-response relationship provided for each symptom) **(more info needed)** Were accepted assessment methods employed? **(more info needed)** Were assessment measures sufficiently sensitive? **(info not provided)** Was the nature and severity of symptoms graded against an explicitly defined scale? (+)Did qualified personnel conduct the exams? (+) Was the timing and frequency of assessment logical? (+) Was raw data provided to permit the reader to arrive at his/her own conclusions? (-) Was individual data reported for each subject? **(no withdrawals)** Are all withdrawals listed, with the reason for withdrawal?
F. Data analysis:	(-) Are the statistical methods used appropriate?

	(-)Are confidence intervals reported? **(more details needed)** Did the investigator appropriately interpret the statistics? **(some)** Are the observed trends statistically significant?
G. Interpretations:	(+) Was the original objective addressed? (-) Are the results novel? (-) Was the design of the study appropriate to investigate the primary objective? (-) Were assumptions reasonable? (-) Are the methods reproducible? **(few)** Did either the study design or the statistical analysis limit confounding variables (*e.g.*, additional chemical exposure, etc.)? (+) Do the conclusions make biological sense? (-) Are the extrapolations reasonable? (+) Is the paper in a peer-reviewed journal? **(No)** Would either the affiliation of the investigative team or sponsor potentially bias the conclusions reached in the document?

Review - Summary:

Discussion of findings:

Emergency room visits correlated positively with concentrations of SO_2. However, statistical significance was not calculated or reported. The deviation from the mean ER visits during the entire year is expressed by variance. Approximately 28% of the variance in the number of ER visits was explained by fluctuations in SO_2. These results were reported as being significant. Due to inadequate analysis of the data, confidence cannot be placed in these findings.

Confidence index:

One limitation of this study, and epidemiological studies in general, is the absence of information about the personal exposure of the individual patient to outdoor or indoor pollutants and/or allergens.

There is very little information on the monitoring stations, SO_2 measurements and sites of the stations.

There is not enough information on how the subjects were diagnosed as having asthma. Outcome misclassification is possible.

There is no analysis of relative risk.

Confidence intervals were not given.

The statistical significance of the associations was not given.

It is not clear how the authors controlled for confounders such as airborne pollen and fungal spores.

The deviation from the mean ER visits was analyzed. SO_2 was reported as accounting for 28% of this variance. There was insufficient detail presented for this analysis.

Some of the assumptions made need more justification.

This study explained the criteria for selecting participants and excluding others. The age group 1-18 was selected in order to exclude children under the age of 1 because of the possible confusion of asthma with viral infections.

Review – Confidence index ranking:

Low